CANCER
TREATMENT REPORTS

June 1986
Volume 70
Number 6
Pages 697-826

U.S.Department of Health
and Human Services
Public Health Service
National Institutes of Health

CANCER
TREATMENT REPORTS

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***

International Cancer Information Center
Susan Molloy Hubbard, ***Director***

Publications Branch
Jean Griffin Baum, ***Chief***

Robert E. Wittes, ***Editor-in-Chief***
Dee Kittner LeRoy, ***Managing Editor***

Editorial Board

Joseph Aisner
Lewis Aronow
Gregory Curt
Richard L. Cysyk
Ross C. Donehower
Michael Friedman
Timothy J. Kinsella
Robert F. Ozols
Carl Pinsky
David G. Poplack
Richard Simon

Advisory Board

Charles M. Balch
Robert C. Bast, Jr
Gianni Bonadonna
Edwin C. Cadman
Giulio J. D'Angio
I. David Goldman
Heine H. Hansen
Simon Kramer
Susan E. Krown
John E. Niederhuber
T. L. Phillips
H. M. Pinedo
Carol S. Portlock
Joseph V. Simone
Samuel A. Wells, Jr

Editorial Staff

Editors:
Elaine Price Beck
Maralyn Farber Berlin
Mary Olvey Cowgill

Editorial Assistant:
Julia Redmond

Manuscript Office:
Sally Bernstein
Kathleen Sager
Olga Sisson

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Cancer Institute, Bethesda, MD 20892 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears on the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

INSTRUCTIONS TO AUTHORS

Title Page
Includes complete title of the manuscript and the full name, affiliation, and address of each author. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Number each page consecutively. Dot-matrix or italicized texts will not be accepted. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use only standard abbreviations; otherwise use full words.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus*: author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, chapter title, book title, city and name of publisher, date of publication, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers published in meeting proceedings should include author; title of paper; title of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs should be clear, glossy prints. If original art work is submitted, it should be done in black ink for good reproduction. Please send 2 copies of each print. Photocopies reproduce poorly. Color photographs or transparencies are acceptable.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section should be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor should be no longer than 3 double-spaced text pages and should be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section should be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge, 100 reprints (20 reprints for Letters to the Editor) will be sent to the senior or specified author about 3 weeks after publication of the article.

Announcements
Announcements of meetings are accepted for publication free of charge. Mail to: Editorial Office, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Institutes of Health, Bethesda, MD 20892.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1987.

METHODOLOGIC GUIDELINES FOR REPORTS OF CLINICAL TRIALS*

1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

2. All patients registered on study should be accounted for. The report should specify for each treatment the number of patients who were not eligible, who died or withdrew before treatment began. The distribution of follow-up times should be described for each treatment, and the number of patients lost to follow-up should be given.

3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

4. In randomized studies, the report should include a comparison of survival and/or other major endpoints for all eligible patients as randomized, that is, with no exclusions other than those not meeting eligibility criteria.

5. The sample size should be sufficient to either establish or conclusively rule out the existence of effects of clinically meaningful magnitude. For "negative" results in therapeutic comparisons, the adequacy of sample size should be demonstrated by either presenting confidence limits for true treatment differences or calculating statistical power for detecting differences. For uncontrolled phase II studies, a procedure should be in place to prevent the accrual of an inappropriately large number of patients, when the study has shown the agent to be inactive.

6. Authors should state whether there was an initial target sample size and, if so, what it was. They should specify how frequently interim analyses were performed and how the decisions to stop accrual and report results were arrived at.

7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be presented in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

8. The patients studied should be adequately described. Applicability of conclusions to other patients should be carefully dealt with. Claims of subset-specific treatment differences must be carefully documented statistically as more than the random results of multiple-subset analyses.

9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

---

* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

Contents

Cancer Treatment Reports
Volume 70, Number 6
June 1986

ORIGINAL ARTICLES

697 Comparative Metabolism of Daunorubicin and 4-Demethoxydaunorubicin in Mice and Rabbits. **M. Broggini, B. Sommacampagna, A. Paolini, E. Dolfini, and M. G. Donelli.** Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy

703 Phase I Study of ICRF-187 in Pediatric Cancer Patients and Comparison of Its Pharmacokinetics in Children and Adults. **J. S. Holcenberg, K. D. Tutsch, R. H. Earhart, R. S. Ungerleider, B. A. Kamen, C. B. Pratt, T. J. Gribble, and D. L. Glaubiger.** Childrens Hospital of Los Angeles, University of Southern California

711 Comparison of Alternating to Nonalternating Chemotherapy Regimens for Non-Small Cell Lung Cancer. **T. J. Meyer, N. W. Pearlman, P. E. Siebert, J. R. Murphy, M. J. White, A. Zaloznik, T. Braun, and P. Rempel.** Veterans Administration Medical Center, University of Colorado Health Sciences Center, Denver General Hospital, and Fitzsimons Army Medical Center

715 Relationship of Glutathione Depletion and Inhibition of Glutathione-*S*-Transferase Activity to the Antimitotic Properties of Estramustine. **K. D. Tew, A. Woodworth, and M. E. Stearns.** Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC

721 Phase I Trial of Menogaril Administered as an Intermittent Daily Infusion for 5 Days. **L. M. Sigman, D. A. Van Echo, M. J. Egorin, M. Y. Whitacre, and J. Aisner.** University of Maryland Cancer Center, University of Maryland School of Medicine, Baltimore

727 Phase II Study of Ifosfamide in Cervical Cancer. **C. A. Meanwell, J. J. Mould, G. Blackledge, F. G. Lawton, N. S. A. Stuart, J. Kavanagh, T. N. Latief, D. Spooner, and A. D. Chetiyawardana.** Queen Elizabeth Medical Centre, Edgbaston, Birmingham, England

731 Phase II Clinical Evaluation of Doxifluridine. **P. Hurteloup, J. P. Armand, P. Cappelaere, R. Metz, P. Kerbrat, R. Keiling, P. Fumoleau, P. Fargeot, S. Schraub, P. Bastit, M. Caudry, C. Cauchie, S. Nasca, E. Pommatau, M. Hayat, P. Chollet, J. Renard, M. Van Glabbeke, and G. Mathé.** European Organization for Research on Treatment of Cancer

739 Northern California Oncology Group Protocol 6G91: Response to Treatment With Radiation Therapy and Seven-Drug Chemotherapy in Patients With Glioblastoma Multiforme. **V. A. Levin, W. M. Wara, R. L. Davis, P. Silver, K. J. Resser, K. Yatsko, S. Nutik, P. H. Gutin, and C. B. Wilson.** Northern California Oncology Group

745 Clinical and Pharmacokinetic Studies of Prolonged Administration of High-Dose Uridine Intended for Rescue From 5-FU Toxicity. **C. J. van Groeningen, A. Leyva, I. Kraal, G. J. Peters, and H. M. Pinedo.** Free University, Amsterdam, The Netherlands

751 Phase II Study of Lonidamine in Patients With Metastatic Renal Cell Carcinoma: A National Cancer Institute of Canada Clinical Trials Group Study. **B. H. Weinerman, E. A. Eisenhauer, J.-G. Besner, C. M. Coppin, D. Stewart, and P. R. Band.** National Cancer Institute of Canada

755 Ip Chemotherapy Employing a Regimen of Cisplatin, Cytarabine, and Bleomycin. **M. Markman, S. Cleary, W. Lucas, R. Weiss, and S. B. Howell.** University of California at San Diego, La Jolla

761 Chemoradiotherapy as Initial Management in Patients With Squamous Cell Carcinoma of the Head and Neck. **D. J. Adelstein, V. M. Sharan, A. S. Earle, A. C. Shah, C. Vlastou, C. D. Haria, S. G. Carter, C. Damm, and J. D. Hines.** Cleveland Metropolitan General Hospital, Case Western Reserve University, Ohio

BRIEF REPORTS

769 Intestinal Cryptosporidiosis: A Complication of Cancer Treatment. **G. M. Mead, J. W. Sweetenham, D. L. Ewins, M. Furlong, and J. A. Lowes.** Southampton General Hospital, Great Britain

771 Phase II Study of Etoposide for Carcinoma of the Prostate. **P. J. Walther, S. D. Williams, M. Troner, F. A. Greco, R. Birch, L. H. Einhorn, and the Southeastern Cancer Study Group.** Duke University Medical Center, Durham, North Carolina

773 Combination Chemotherapy With Mitomycin and Vindesine in Advanced Non-Small Cell Lung Cancer: A Pilot Study by the Lung Cancer Working Party (Belgium). **J. P. Sculier, J. Klastersky, J. P. Dumont, G. Vandermoten, P. Rocmans, P. Libert, P. Ravez, D. Becquart, P. Mommen, and O. Dalesio.** Lung Cancer Working Party (Belgium)

777 High-Dose Oral Medroxyprogesterone Acetate in Heavily Pretreated Patients With Metastatic Breast Cancer. **P. E. Goss, S. Ashley, T. J. Powles, and R. C. Coombes.** Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, England

781 Phase II Trials of 5-FU, Doxorubicin, and Cisplatin in Advanced, Measurable Adenocarcinoma of the Lung and Stomach. **E. L. Cazap, C. Gisselbrecht, F. P. Smith, R. A. Estevez, C. A. Alvarez, C. Lagarde, A. Hannois, S. Ahmed, P. S. Schein, and P. V. Woolley.** Universidad del Salvador, Buenos Aires, Argentina

785 Modified Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, and Cytarabine (COMLA) in Intermediate- and High-Grade Lymphoma: An Effective Short-Course Regimen. **M. R. Baer, R. S. Stein, J. P. Greer, S. N. Wolff, J. D. Hainsworth, and J. M. Flexner.** Vanderbilt University Hospital and Veterans Administration Medical Center, Nashville, Tennessee

789 Use of Cisplatin, Doxorubicin, and Cyclophosphamide To Treat Advanced and Recurrent Adenocarcinoma of the Endometrium. **K. C. Hancock, R. S. Freedman, C. L. Edwards, and F. N. Rutledge.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

CLINICAL TRIALS SUMMARIES

793 Phase II Study of the Platinum Analog TNO-6 in Patients With Advanced Ovarian Cancer. **N. Colombo, E. Sartori, F. Landoni, G. Favalli, L. Vassena, L. Zotti, E. Materman, C. R. Franks, S. Pecorelli, and C. Mangioni.** Ospedale S. Gerardo, Monza, Italy

795 Combination Chemotherapy With Vindesine and Cisplatin for Refractory Small Cell Bronchogenic Carcinoma. **D. F. Chiuten, D. T. Carr, H. M. Dhingra, W. K. Murphy, G. Spitzer, T. Umsawasdi, G. P. Bodey, and M. Valdivieso.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

797 Abbreviated Phase I Clinical Trial of Iv Mitozolomide. **R. A. Joss, H. J. Ryssel, A. K. Bischoff, A. Goldhirsch, and K. W. Brunner.** University of Bern, Inselspital, Switzerland

799 Phase II Trial of Aclarubicin in Epidermoid Carcinoma of the Head and Neck. **A. A. Carugati, A. J. Olivari, D. M. Campos, R. Pradier, and P. V. Woolley.** University of Buenos Aires, Argentina

801 Phase II Evaluation of Etoposide in Refractory Multiple Myeloma: A Southeastern Cancer Study Group Trial. **J. P. Gockerman, A. A. Bartolucci, M. O. Nelson, H. Silberman, E. Velez-Garcia, and R. Stein.** Southeastern Cancer Study Group

803 Phase II Evaluation of Aclarubicin in Lung Cancer: A Southeastern Cancer Study Group Trial. **B. S. Kramer, R. Birch, J. P. Gockerman, A. Greco, and K. Prestridge.** Southeastern Cancer Study Group

805 Epirubicin in Non-Oat Cell Lung Cancer—Response Rates and the Importance of Immunopathology: A Northern California Oncology Group Study. **F. J. Meyers, R. D. Cardiff, R. Quadro, M. Gribble, M. Kohler, V. Medrano, E. P. Mitchell, R. Shiffman, and L. William.** Northern California Oncology Group

807 Phase II Study of Vindesine in the Treatment of Pediatric Patients With Solid Tumors: A Report From the Childrens Cancer Study Group. **M. D. Krailo, W. Krivit, H. Sather, and D. Hammond.** Childrens Cancer Study Group

811 Tamoxifen in Advanced and Recurrent Uterine Sarcomas: A Phase II Study. **Gynecological Group, Clinical Oncological Society of Australia and The Cancer Institute, Melbourne**

813 Phase II Trial of *N*-Methylformamide in Patients With Advanced Colorectal Carcinoma. **K. Tauer,**

N. Kemeny, E. Cheng, P. Hollander, and N. Geller. Memorial Sloan-Kettering Cancer Center, New York, New York

815 Weekly Low-Dose Doxorubicin in Advanced Breast Cancer. **P. Specenier and J. Thomas.** University Hospital St. Rafaël, Leuven, Belgium

LETTERS

817 Intrinsic Versus Acquired Drug Resistance. **W. J. Mackillop.** Kingston Regional Cancer Centre and Queen's University, Ontario, Canada

819 Reply. **J. H. Goldie and A. J. Coldman.** Cancer Control Agency of British Columbia, Vancouver, Canada

819 Simple Method for the Administration of High-Dose Etoposide During Autologous Bone Marrow Transplantation. **H. M. Lazarus, R. J. Creger, and D. Diaz.** Case Western Reserve University, University Hospitals of Cleveland, Ohio

820 Dose-Limiting Myelosuppression Associated With High-Dose Cisplatin (200 mg/m^2) in Hypertonic Saline. **D. R. Gandara, R. Mansour, H. Wold, and C. George.** Letterman Army Medical Center, San Francisco, University of California, Davis, and Martinez Veterans Administration Medical Center

823 ANNOUNCEMENTS

Comparative Metabolism of Daunorubicin and 4-Demethoxydaunorubicin in Mice and Rabbits[1,2]

Massimo Broggini, Beatrice Sommacampagna, Angela Paolini, Ersilia Dolfini, and Maria Grazia Donelli[3,4,*]

In the present study, the biodisposition of 4-demethoxydaunorubicin (4DDM) and its 13-dihydrometabolite was compared with daunorubicin (DM) and its metabolite in rabbit serum, and the results were considered in the light of this DM analog's pharmacokinetic behavior in mice. In rabbit serum, the levels of the 13-OH derivative of both DM and 4DDM (daunorubicinol and 4-demethoxydaunorubicinol) were higher than in mice. In vitro metabolic studies with mouse and rabbit cytosol indicated that the hepatic metabolism was quantitatively important for both analogs (70%–90% for DM and 4DDM was reduced to the 13-OH metabolite), but the rabbit had a much higher specific capacity to metabolize these compounds. DM seemed a better substrate for cytoplasmic aldoketoreductase, the enzyme affinity in rabbits being three times higher than for 4DDM. Cytotoxicity studies in vitro showed that 4-demethoxydaunorubicinol, unlike daunorubicinol, was as cytotoxic as the parent compound, and this suggests this metabolic step does not inactivate 4DDM but contributes to its high and long-lasting biological activity in vivo. [Cancer Treat Rep 70:697–702, 1986]

4-Demethoxydaunorubicin (4DDM), a daunorubicin (DM) derivative lacking the methoxyl group in position 4 of the aglycone, is one of the anthracycline analogs which have revealed interesting and promising features. Its greater potency and activity against some experimental leukemias and lymphomas, together with its lower cardiotoxicity (1–4) and efficacy by the oral route (5), suggest that this analog may be superior to its parent compound and justify the interest of investigators and clinicians.

Previous studies by our group on 4DDM pharmacokinetics (6) in tumor-bearing mice have shown that, after equitoxic treatment, 4DDM is cleared from plasma slower than DM. In tissues, including the tumor, 4DDM exposure is twice as high, with correspondingly longer half-lives than its parent compound. Moreover, this demethoxy analog appeared somewhat more selective than DM, as the relative capacity of tumor tissue to accumulate 4DDM seemed higher than that of other organs such as heart and spleen, which are reportedly targets of toxicity. We also reported that, in mice, 4DDM seems to be metabolized to its major metabolite, 4-demethoxydaunorubicinol (4DDMol), to a much lower extent than DM, amounting in serum to about 20% of the concentration of the native compound as compared to daunorubicinol (DMol), which is > 100% of the DM concentration. However, in humans, other authors have shown that the levels of 4DDMol in serum appear higher and its disappearance rate lower than 4DDM, suggesting extensive formation and prolonged retention of 4DDMol (7,8). Moreover, conversely to the DM metabolite DMol, observations by Casazza et al (9) suggest a marked cytotoxicity of the 4DDM 13-OH metabolite.

The reported interspecies differences and the suggested cytotoxicity of 4DDMol prompted us to extend our investigation by comparatively analyzing the pharmacokinetic behavior and the metabolism of DM and 4DDM in different species, with the aim of clarifying the role of metabolism in their biological activity.

The pharmacokinetics of the two DM analogs and their reduced metabolites was investigated in the rabbit, a species very similar to man with regard to the activity of aldoketoreductase, which is responsible for anthracycline metabolism (10,11). In vitro hepatic production of the metabolites obtained by cytoplasmic carbonyl reductase was studied in mice and rabbits. The marked cytotoxicity of 4DDMol, confirmed in our studies on human neoplastic cells in culture, suggests that 4DDM biological activ-

[1]Received Mar 18, 1985; revised Aug 6, 1985; accepted Dec 2, 1985.

[2]Supported in part by Farmitalia-Carlo Erba, Milan, Italy; by CNR (Progetto Finalizzato "Oncologia"); and by a contribution from the Italian Association for Cancer Research, Milan.

[3]Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

[4]We thank Drs. A. M. Casazza and F. Giuliani for fruitful advice and discussion, and Prof. F. Arcamone and Dr. Penco for providing daunorubicin, 4-demethoxydaunorubicin, and their metabolites.

[*]*Reprint requests to:* Maria Grazia Donelli, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea, 62, 20157 Milan, Italy.

ity is also ascribable to the bioproduct of its major metabolic step.

MATERIALS AND METHODS

Animals and Drugs

The following animal species were used: C57BL/6 female mice (20 ± 2 g of body weight) and New Zealand rabbits (2.5 ± 0.1 kg of body weight); the animals were obtained from Charles River, Italy. DM, 4DDM, and their reduced metabolites DMol and 4DDMol were kindly supplied by Farmitalia-Carlo Erba, Milan, Italy. The drugs were dissolved in saline for animal treatment and in methanol for the standard solutions for biochemical assay.

Pharmacokinetic Studies

Pharmacokinetic studies in rabbits were conducted by injecting animals with DM (5 mg/kg iv) or 4DDM (2 mg/kg iv) at equitoxic doses. Blood samples were obtained from an ear vein 1, 5, 10, 20, and 30 minutes and 1, 2, 4, 6, and 24 hours after administration; three rabbits per group were used. Plasma, obtained by centrifuging heparinized blood samples for 30 minutes at 2800 × *g*, was stored at -20°C until analysis. Plasma pharmacokinetic parameters were determined for each rabbit by a general nonlinear fitting procedure using the least-squares criterion, with the aid of a microcomputer program (12).

Metabolic Studies In Vitro

Livers from mice and rabbits were homogenized in 1.15% KCl, pH 7.4, and centrifuged at 9000 × *g* for 30 minutes at 4°C. The supernatants were removed and centrifuged for 60 minutes at 105,000 × *g*. The cytosol fraction from the high-speed supernatants was used. Incubations were carried out at 37°C and pH 7.4 in the presence of DM or 4DDM at concentrations ranging from 1 to 50 μM (1, 2, 5, 10, 20, and 50 μM), G_6P dehydrogenase (5 μl × sample), G_6P 10 mM, NADP 0.3 mM, and $MgCl_2$ 50 mM in a final volume of 5 ml. The reaction was stopped by adding 8 ml of ice-cold chloroform:isopropyl alcohol (1:1), and the disappearance of DM or 4DDM, with the production of 13-OH metabolite, was then analyzed in samples of the incubation mixtures.

Rates of metabolite production and substrate disappearance were linear with time for 30 minutes and proportional to cytosol protein concentration. Protein content was assessed by the method of Lowry et al (13).

Cytotoxicity Studies In Vitro

The cell line M14, derived from human metastatic nodules, was kindly provided by Dr. G. Zupi, Regina Elena Institute, Rome, Italy. The cell line was maintained in our laboratory in RPMI 1640 Gibco medium (Glasgow, Scotland), supplemented with 10% fetal calf serum plus 1-glutanine in Corning flasks. For subcultures, the monolayers were rinsed once with phosphate-buffered saline (Ca^{++}- and Mg^{++}-free) and incubated with 0.5 mM EDTA at 37°C. The cells were dispersed in RPMI 1640 plus 10% fetal calf serum and counted in a model B Coulter counter. Subcultures were obtained by seeding about 7×10^6 cells in 5 ml of culture medium in Corning flasks.

The experiments were made when subcultures were in the exponential growth phase (2–2.4 × 10^5 cells/cm^2). About 1.2×10^5 cells were seeded in growth medium in tissue culture cluster plates (24 wells, 2-cm^2 area/well) and incubated at 37°C in 5% CO_2, 95% air, for 24 hours. Treatment consisted of adding the different concentrations of the antineoplastic agents (0.018, 0.18, and 1.8 nmols/ml) and incubating for 24 hours; at the end of incubation, the cell monolayers were washed with phosphate-buffered saline and released by 4-minute incubation in EDTA. The cell suspensions were counted and the cytotoxic activity was calculated by measuring the inhibition of growth or destruction of the M14 cells (14). The percentage of growth was calculated compared to the increase in the number of control cells. The percentage of destruction was calculated compared to the baseline value. The experiments were repeated twice, each group consisting of five samples.

Analytical Assay

DM, 4DDM, and their reduced metabolites DMol and 4DDMol were determined by high-performance liquid chromatography with fluorimetric detection. Drugs were extracted from plasma or liver supernatants (105,000 × *g*) as described in detail elsewhere (6). Recovery was 85% ± 3% and sensitivity was 5 ng/ml of fluids.

RESULTS

Pharmacokinetics of DM and 4DDM in the Rabbit

To extend the pharmacokinetic investigation of DM and 4DDM to an animal species more similar to humans from the point of view of anthracycline metabolism (10,11), the in vivo disposition of these compounds given at equitoxic doses and their reduced metabolites was investigated in the rabbit.

DM disappeared from the circulation very rapidly, and was no longer detectable as early as 120 minutes after treatment (fig 1). Concomitantly, the levels of the 13-OH derivative were sustained and persistent, with ten times more metabolite than the parent drug observable in serum as early as 1 minute after drug injection. 4DDM was still present in serum 6 hours after treatment, and concentrations of its reduced metabolite largely exceeded those of the parent drug, although they were proportion-

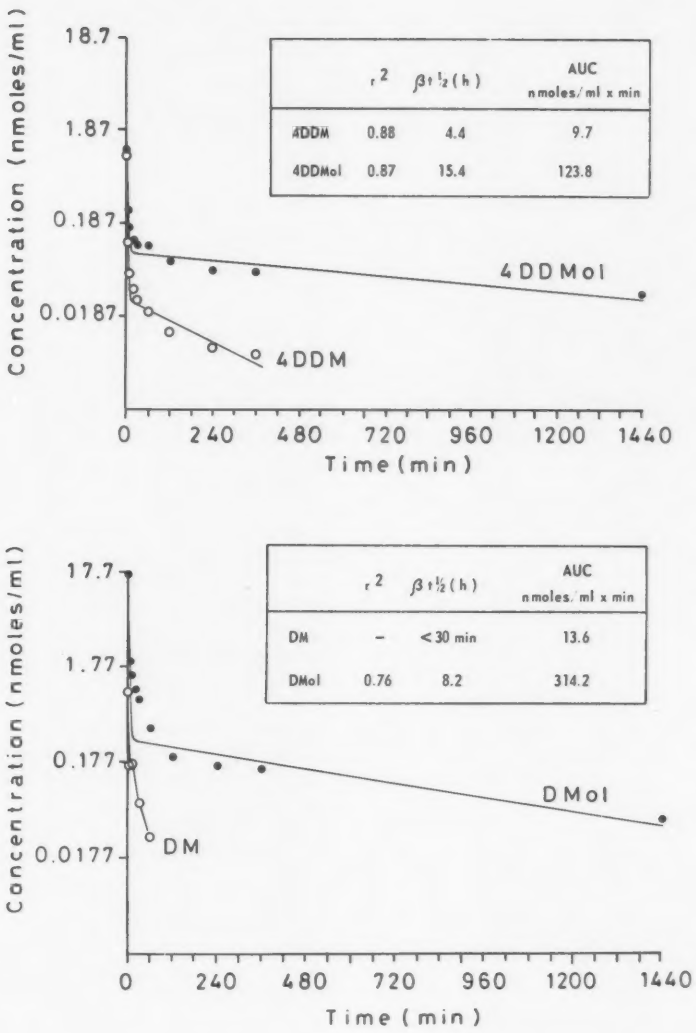


FIGURE 1.—Serum levels of 4DDM and 4DDMol (2 mg/kg iv) and DM and DMol (5 mg/kg iv) in rabbits. ○——○ = parent drug; ●——● = reduced metabolite.

ally lower than DMol. The presence of 4DDMol in the circulation is long-lasting, as its elimination half-life was 15.4 versus 8.2 hours for DMol and 4.4 hours for unchanged 4DDM. Although the theoretical curve for DMol presented in figure 1 does not seem to fit the experimental data very well, the value of the coefficient $r^2 = 0.76$ confirms the significance of the correlation in our two-compartment model.

In Vitro Metabolism Studies

The disappearance of unchanged DM or 4DDM and the concomitant production of the 13-dihydroderivative, the major metabolite obtained by carbonyl reduction with aldoketoreductase of the liver, was first investigated in vitro. Liver cytosol from mice and rabbits was incubated with DM or 4DDM at 5 μM for 60 minutes.

TABLE 1.—In vitro metabolism of DM and 4DDM in mice and rabbits*

	13-OH derivative produced (% of the substrate)†		K_m (μM)‡		V_{max} (nmols/min/mg)‡	
	DMol	4DDMol	DM	4DDM	DM	4DDM
Mouse (60 mg of liver/ml)	97 ± 1.0	100 ± 0.8	111 ± 9	146 ± 12	0.67 ± 0.03	0.68 ± 0.02
Rabbit (0.8 mg of liver/ml)	83 ± 3	70 ± 1	9.7 ± 0.6	33.1 ± 3.5	1.45 ± 0.04	3.00 ± 0.25

*Values = mean ± SE.

†Liver cytosol was incubated for 60 mins in the presence of cofactors and DM or 4DDM (5 μM). Values are the average of 4 samples.

‡Apparent K_m and V_{max} were calculated from Woolf Plot s/v or S. Liver cytosol was incubated with different drug concentrations for 30 mins (*see* Materials and Methods section). Values are the average of 3 experiments.

The metabolism of these analogs to DMol or 4DDMol was quantitatively important for both compounds, amounting to 70%-90% of the initial in vitro drug concentration (table 1). Rabbits showed a much higher metabolic rate than mice, as a 100 times lower enzyme concentration was required to reach the same metabolite production.

The specific capacity of the liver to metabolize DM or 4DDM was then compared in enzymatic kinetic studies, utilizing the aldoketoreductase of the cytosol fraction of the liver of mice and rabbits. DM appears to be a better substrate than 4DDM for the enzyme, as the lower K_m values found for DM, particularly in the rabbit (9.7 ± 0.6 μM for DM vs 33.1 ± 3.5 μM for 4DDM), indicate a higher affinity of aldoketoreductase for the parent compound. The higher V_{max} values of the enzyme for 4DDM are not representative of the in vivo situation, where at therapeutic drug doses saturation of the substrate never occurs.

In Vitro Cytotoxicity Studies

The cytotoxicity of the reduced metabolites of DM and 4DDM, whose production and presence in the body are so important, was investigated in vitro on human tumor cell lines (table 2). Unlike DMol, 4DDMol was highly cytotoxic, at least as much as the parent compound and more than DM itself. While DM results in cell destruction only at the higher dose, 4DDM and 4DDMol destroyed the cultured cells at all concentrations tested (0.018–1.8 nmols/ml).

TABLE 2.—In vitro cytotoxicity of DM, 4DDM, and their reduced metabolites DMol and 4DDMol*

	nmols/ml	% inhibition	% destruction
DM	0.018	20	0
	0.18	80	0
	1.8	100	50
DMol	0.018	18	0
	0.18	46	0
	1.8	85	0
4DDM	0.018	100	22
	0.18	100	44
	1.8	100	70
4DDMol	0.018	100	24
	0.18	100	52
	1.8	100	80

*1.1×10^5 cells/ml of human melanoma M14 in culture treated for 24 hrs. Values are the average of 2 experiments, 5 samples/point each.

DISCUSSION

The present findings confirm that removal of the C_4-methoxy group in the aglycone moiety of the DM molecule substantially changes its pharmacokinetic, metabolic, and chemotherapeutic profile, as suggested in our previous work. The pharmacokinetic study in rabbits indicated that, as observed in mice (6), 4DDM takes much longer to disappear from serum than DM ($\beta t_{1/2}$ of 4DDM is 4.4 hrs vs < 30 mins for DM). However, in contrast with mice, rabbit serum levels of 4DDMol were of the same order as DMol levels and much higher and prolonged than those of 4DDM, suggesting that in this animal species the biodisposition of this anthracycline analog and its reduced metabolite is similar to the pharmacokinetic profile in humans (7,8).

The marked, prolonged presence of hydroxylated metabolites of 4DDM in the serum of humans and rabbits confirms that this DM analog, as widely described for this class of compounds (15-17), undergoes large-scale carbonyl reduction, although to a lower extent than DM, in good agreement with its slower serum clearance (fig 1 and table 3). This extensive hydroxylation in vivo is confirmed in our studies of hepatic metabolism in vitro, where the metabolizing capacity of two animal species was investigated. Although aldoketoreductases of rabbits and mice in liver cytoplasmic extracts have been reported to be numerous and to show optimal activity at different pH (11,18), the in vitro metabolism of DM and 4DDM in these species was tested in the same physiological conditions at pH 7.4 in order to permit comparison of the in vitro results with the in vivo metabolism. Cytosol of both species metabolized as much as 70%-90% of both substrates in the presence of saturation concentrations. As the in vivo situation is usually far from saturation of enzymes, it was important to establish the specific affinity of cytoplasmic aldoketoreductase for these substrates in conditions as close as possible to the in vivo

TABLE 3.—Summary of the in vitro and in vivo metabolism of 4DDM compared to DM in mice and rabbits*

	Serum AUC Metabolite / Parent drug		Serum $\beta t_{1/2}$ (hrs)				K_m (μM)	
	DM	4DDM	DM	DMol	4DDM	4DDMol	DM	4DDM
Mouse	2.20	0.26	4.6	13.9	23.6	ND	111	146
Rabbit	23.20	12.80	< 30 mins	8.2	4.4	15.4	9.7	33.1

*Animals were injected as follows: DM—15 mg/kg iv in mice, 5 mg/kg iv in rabbits; 4DDM—3 mg/kg iv in mice, 2 mg/kg iv in rabbits. For details, *see* footnotes to table 1 and ref 6. AUC = area under the curve; ND = not determined.

situation. Once more, DM seemed a better substrate than 4DDM, particularly in rabbits, where the K_m of the enzyme for DM was three times that for its demethoxy analog. If the in vitro and in vivo metabolism of DM and 4DDM in mice and rabbits is comparatively observed (table 3), it is clearly evident that the metabolic ratio is in favor of DM in both species. Indeed, the overall availability of the 13-OH derivative in serum relative to its parent compound, as reflected by the ratio between area under the curve values of metabolite and parent drug, was much lower for 4DDM, in good agreement with the lower enzyme affinity (higher K_m values) and the higher serum half-life of this DM analog. However, variations in enzyme affinity for either substrate in mice and rabbits do not always account for the metabolite disposition observed in vivo; for example, 4DDMol levels in serum of mice were much lower than might be expected on the basis of the enzyme kinetic studies, although this disparity in the relative metabolite levels for both analogs was not marked in tissues (6). This is further evidence that no direct relationship can be established between in vivo and in vitro results, as the in vivo condition is far more complex and many factors besides metabolism may account for the large interspecies differences in the pharmacokinetic profiles of many compounds (19). For anthracycline compounds in particular, metabolism of the hematological components (20,21), rate and extent of tissue uptake which is linearly related to the hydrophilic nature of the drugs and their metabolites (22), rate and extent of elimination, and so on may vary independently for the various drugs in different animal species.

Although the differences between mice and rabbits in the in vivo and in vitro metabolism of DM are surprisingly of the same order (rabbits show an enzyme activity and an amount of 13-OH derivative about ten times that in mice), for 4DDM the very low levels of 4DDMol in the serum of mice result in ratios of unchanged drug and metabolite reversed in the two species. This finding, together with the existence of different classes of aldoketoreductases in mice and rabbits (11,18), again suggests that experimental results in rodents for anthracycline antibiotics cannot always be extrapolated to the human situation and different analogs may behave differently in the various species.

In view of the fact that, in mice and to a greater extent in rabbits, the exposure of tissues to the 13-OH derivatives of DM and 4DDM is quantitatively high and prolonged, the extent to which these metabolites contribute to the biological effects of these compounds is an important question. Our in vitro studies on the comparative cytotoxicity of these anthracycline compounds and their reduced metabolites on human tumor cell lines in culture indicate that 4DDMol, unlike DMol, is highly cytotoxic, with potency comparable to the parent compound, as already shown in murine Hela cells and in P388 leukemia (9). The metabolic pathway of carbonyl reduction, responsible for the inactivation of DM, therefore seems an important step in exploitation of the biological effects of the 4DDM analog. The ultimate activity of this compound should be attributed not only to its higher potency and larger bioavailability than DM, but also to the high cytotoxicity of its main metabolite, whose contribution is obviously more important in animal species with higher production, such as rabbits and humans.

REFERENCES

1. CASAZZA AM. Experimental evaluation of anthracycline analogs. Cancer Treat Rep 63:835-844, 1979.
2. CASAZZA AM, PRATESI G, GIULIANI F, ET AL. Antileukemic activity of 4-demethoxydaunorubicin in mice. Tumori 66:549-564, 1980.
3. CASAZZA AM, DI MARCO A, BONADONNA G, ET AL. Effects of modifications in position 4 of the chromophore or in position 4′ of the aminosugar on the antitumor activity and toxicity of daunorubicin and doxorubicin. *In* Anthracyclines: Current Status and New Developments (Crooke ST, and Reich SD, eds). New York, Academic Press, 1980, pp 403-430.
4. ZANON PL, LAMBERTENGHI-DELILIERS G, POZZOLI EF, ET AL. Selective mitochondrial alterations induced by a single dose of daunorubicin or 4-demethoxydaunorubicin in mouse ventricular myocardium. Tumori 66:27-34, 1980.
5. DI MARCO A, CASAZZA AM, and PRATESI G. Antitumor activity of 4-demethoxydaunorubicin administered orally. Cancer Treat Rep 61: 893-894, 1977.
6. BROGGINI M, ITALIA C, COLOMBO T, ET AL. Activity and distribution of iv and oral 4-demethoxydaunorubicin in murine experimental tumors. Cancer Treat Rep 68:739-747, 1984.
7. BERMAN E, WITTES RE, LEYLAND-JONES B, ET AL. Phase I and clinical pharmacology studies of intravenous and oral administration of

4-demethoxydaunorubicin in patients with advanced cancer. Cancer Res 43:6096-6101, 1983.
8. MORO E, BELLOTTI V, JANNUZZO MG, ET AL. High-performance liquid chromatographic method for pharmacokinetic studies on the new anthracycline 4-demethoxydaunorubicin and its 13-dihydro-derivative. J Chromatogr 274:281-287, 1983.
9. CASAZZA AM, BARBIERI B, FUMAGALLI A, ET AL. Biologic activity of 4-demethoxy-13-dihydrodaunorubicin. Proc Am Assoc Cancer Res 24:251, 1983.
10. LOVELESS H, ARENA E, FELSTED RL, ET AL. Comparative mammalian metabolism of Adriamycin and daunorubicin. Cancer Res 38:593-598, 1978.
11. FELSTED RL, and BACHUR NR. Human liver daunorubicin reductases. Prog Clin Biol Res 114:291-305, 1982.
12. SNEDECOR GW, and COCHRAN WG. Statistical Methods, 6th ed. Ames, IA, Iowa University Press, 1967.
13. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275, 1951.
14. MORASCA L, and LEONARDI A. Méthode rapide pour l'évaluation quantitative de l'activité cytotoxique en culture de tissus par une numeration électronique des cellules. Rev Fr Etud Clin Biol 10:759-760, 1965.
15. BACHUR NR. Daunorubicinol, a major metabolite of daunorubicin: isolation from human urine and enzymatic reactions. J Pharmacol Exp Ther 177:573-578, 1971.
16. BACHUR NR, and GEE M. Daunorubicin metabolism by rat tissue preparations. J Pharmacol Exp Ther 177:567-572, 1971.
17. BACHUR NR. Adriamycin (NSC-123127) pharmacology. Cancer Chemother Rep Part 3, vol 6 (2):153-158, 1975.
18. AHMED NK, FELSTED RL, and BACHUR NR. Heterogeneity of aldo-keto reductases. Fed Proc 36:723, 1977.
19. BONATI M, LATINI R, TOGNONI G, ET AL. Interspecies comparison of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. Drug Metab Rev 15:1355-1383, 1984.
20. HUFFMAN DH, and BACHUR NR. Daunorubicin metabolism by human hematological components. Cancer Res 32:600-605, 1972.
21. VASANTHAKUMAR G, and AHMED NK. Uptake and metabolism of daunorubicin by human myelocytic cells. Cancer Chemother Pharmacol 15:35-39, 1985.
22. BACHUR NR, STEELE M, MERIWETHER WD, ET AL. Cellular pharmacodynamics of several anthracycline antibiotics. J Med Chem 19:651-656, 1976.

Phase I Study of ICRF-187 in Pediatric Cancer Patients and Comparison of Its Pharmacokinetics in Children and Adults[1,2]

John S. Holcenberg,[3,*] Kendra D. Tutsch,[4] Robert H. Earhart,[5] Richard S. Ungerleider,[6] Barton A. Kamen,[7] Charles B. Pratt,[8] T. John Gribble,[9] and Daniel L. Glaubiger[6,10]

A phase I study of ICRF-187 as a 2-hour iv infusion daily for 3 days was conducted in 46 evaluable pediatric patients. The maximum tolerated dose was 3500 mg/m²/day × 3 based on changes in hepatic function and coagulation abnormalities encountered when larger dosages were administered. One patient with acute lymphocytic leukemia achieved a complete remission and four cleared the blood of lymphoblasts. No other objective responses were seen. Pharmacokinetic analysis showed that the children had a larger volume of distribution per kilogram of body weight in the central compartment and total body and a more rapid total-body clearance than adults. These parameters can explain only part of the increased tolerance of children to ICRF-187. [Cancer Treat Rep 70:703–709, 1986]

ICRF-187 is the (+)-enantiomer of the racemic mixture called razoxane (ICRF-159). Oral administration of ICRF-159 produced clinical responses in non-Hodgkin's lymphoma, colorectal carcinoma, non-oat cell carcinoma, Kaposi's sarcoma, and childhood leukemia (1,2). The drug is also of interest because of its antimetastatic effects, its synergism with other antitumor compounds and radiotherapy, and its ability to protect the heart from anthracycline toxicity in animal models (3–6). The oral preparation has limited bioavailability, especially in large doses (7). To overcome this problem, a parenteral preparation was sought. Repta et al (8) showed that the pure enantiomers are fivefold more water soluble than the racemate. Subsequently, animal models showed that the enantiomers had antitumor activity.

Adult phase I trials have been conducted with several dosage schedules. The dose-limiting toxic effects have been granulocytopenia and thrombocytopenia. In adults, the maximum tolerated dose (MTD) for short iv infusions has been 1000–1250 mg/m²/day × 3 or 800–1350 mg/m²/day × 5, depending on the extent of prior treatment (9,10). In contrast, only 666–1000 mg/m² is tolerated as a 48-hour constant infusion (11). We started a pediatric phase I study identical to the adult daily × 3 schedule (9). No myelosuppression was seen at the adult MTD. Slow escalation continued until a new toxic effect, transient hepatic dysfunction, was noted at doses about three times the adult MTD. Because of this great difference in MTD between children and adults, pharmacokinetic studies were performed and compared with the adult data (12). The ICRF-187 pharmacokinetics between the first 2-hour infusion in the pediatric study was compared with that of the single infusions of 0.5- and 8-hour duration in the adult study. These results show a correlation between the volume of distribution of ICRF-187 and the MTDs in children and adults. A preliminary report of the pharmacokinetic data has been published (13).

MATERIALS AND METHODS

Eligibility Criteria

Patients ⩽ 21 years of age with pathologic confirmation of malignancy that was resistant to conventional

[1]Received May 2, 1985; revised Nov 12, 1985; accepted Dec 4, 1985.

[2]Supported by the T. J. Martell Foundation for Research on Leukemia and Cancer; the Milwaukee Athletes Against Childhood Cancer; the American Lebanese Syrian Associated Charities; and by Public Health Service grant CA-23099 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Department of Pediatrics, Childrens Hospital of Los Angeles, University of Southern California.

[4]Department of Human Oncology, University of Wisconsin Medical School, Madison.

[5]The Upjohn Company, Kalamazoo, MI.

[6]Clinical Investigations Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[7]Department of Pediatrics, University of Texas, Dallas.

[8]St. Jude Children's Research Hospital, Memphis, TN.

[9]University of New Mexico, Albuquerque.

[10]We thank Drs. Vassilios Avramis, William Evans, and David D'Argenio for helpful suggestions, and Amy Plagge and Patrice Turner for careful preparation of the manuscript.

[*] *Reprint requests to:* John S. Holcenberg, MD, Division of Hematology/Oncology, Childrens Hospital of Los Angeles, PO Box 54700, Los Angeles, CA 90054-0700.

therapy were eligible for study. An estimated life expectancy of at least 4 weeks was required. Patients must have recovered from the toxic effects of all prior chemotherapy and radiotherapy prior to entering this study. Solid tumor patients without bone marrow invasion by tumor were required to have an absolute granulocyte count > 1500/mm^3, platelet count > 100,000/mm^3, adequate liver function (bilirubin < 2.0 mg/dl, normal prothrombin time), adequate renal function (creatinine < 1.5 mg/dl or creatinine clearance > 80 ml/minute/1.7 m^2), and normal metabolic parameters (serum electrolytes, blood sugar, calcium, and phosphorus). Patients with leukemia or bone marrow tumor invasion were eligible despite granulocytopenia, anemia, and thrombocytopenia if this marrow suppression was due to the leukemic or tumor invasion. Signed informed consent indicating the investigational nature of this study was obtained.

Drug Formulation and Administration

ICRF-187 was supplied by the National Cancer Institute (Bethesda, MD) as a sterile lyophilized powder in 30-ml vials containing 250 mg of the compound. Each vial was reconstituted in 25 ml of sodium chloride, UPS, for injection. Because of limited solubility, the drug was further diluted to a concentration < 10 mg/ml with a 5% dextrose solution with or without electrolytes and administered over 2 hours. The same dosage was given on 3 consecutive days, usually in the outpatient clinic.

The initial daily × 3 dose was 500 mg/m^2. When we learned that the adult MTD on this schedule was 1250 mg/m^2, we escalated to 80% of that dose. Further dose escalations were made in 250–500-mg/m^2 increments. Patients with leukemia and solid tumor were escalated in parallel. There was no dose escalation in individual patients. Patients were treated for additional cycles at 3-week intervals if they tolerated the therapy and had no progression of their malignancies. No other cancer chemotherapy or radiotherapy was administered during the study.

Patients were monitored by physical examination, blood cell counts with differential, platelet count, urinalysis, BUN, creatinine, serum calcium, phosphorus, magnesium, electrolytes, uric acid, blood sugar, SGOT, bilirubin, alkaline phosphatase, albumin, globulin, prothrombin time, and partial thromboplastin time. Prior to a study a bone marrow was obtained in patients with leukemia and appropriate diagnostic studies were obtained to evaluate the extent of disease in patients with solid tumor. These tests were repeated as needed to evaluate response.

Pharmacokinetic Studies

Blood samples were obtained before the infusion; at 10, 20, 40, 60, 80, and 120 minutes during the infusion; and at 10, 20, 40, 60, and 120 minutes and at 3, 6, 8, and 15 hours after the infusion. Approximately 5 ml of blood was allowed to clot at 0°C–5°C, centrifuged, and the serum separated and frozen. A 24-hour urine collection was started at the beginning of the infusion and kept on ice. An aliquot was frozen. The concentration of ICRF-187 was determined by high-pressure liquid chromatography, according to a published method (12). Postinfusion data were fitted using the following two-compartmental model (14) and calculated as previously described (12).

The zero-time intercepts A′ and B′ were calculated from:

$$A' = \frac{R\alpha T}{1-e^{-\alpha T}}; B' = \frac{S\alpha T}{1-e^{-\beta T}},$$

where T is the infusion time, and R, S, α, and β are the parameters calculated from the postinfusion data by Autoan-Nonlin analysis and Estrip (15,16). A weighting factor of $1/C$ was used for the nonlinear least-squares analysis. Both methods gave nearly identical estimates. The microconstants k_{12}, k_{21}, and k_{10}; volume of distribution (Vb); volume of the central compartment (Vc); and clearance (Cl) were computed by standard methods.

The data were also analyzed by the following noncompartmental methods (17,18):

$$Vss = \text{total dose}\left(\frac{AUMC}{AUC^2} - \frac{T}{2AUC}\right), \quad MRT = \frac{AUMC}{AUC} - \frac{T}{2},$$

where the total area under the serum concentration curve (AUC) and the first moment of the serum concentration-time curve (AUMC) were calculated by the trapezoidal rule extrapolating the last sampling time to infinity by addition of the terms

$$\frac{C_{last}}{\beta} \text{ and } \frac{C_{last}\, t_{last}}{\beta} + \frac{C_{last}}{\beta^2}$$

to AUC and AUMC, respectively. Vss is the volume of distribution at steady state and T is the infusion time. MRT is the mean resident time corrected for the infusion time.

The previously published pharmacokinetic data for adults were reanalyzed. The noncompartmental analysis requires data during the infusion for accurate calculation of the AUC and AUMC. These data were not available for some of the adult and pediatric patients. Therefore, simulated data points at 15, 30, and 60 minutes and at 60-minute increments until the end of infusion were calculated from the two-compartment analysis data on each patient according to the following equation (14):

$$C(t) = \frac{Dose}{T \cdot Vc}\left(\frac{1 + \beta - k_{10}e^{-\alpha t} + k_{10} - \alpha e^{-\beta t}}{\alpha - \beta}\right)$$

In patients for whom serum concentration values were measured during the infusion, the calculated and meas-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	48
Evaluable	46
Ineligible (excluded from analysis)	2*
Age range (yrs): 2-21†	
Sex	
Male	21
Female	25
Diagnosis	
Acute lymphocytic leukemia	17
Acute myelocytic leukemia	3
Rhabdomyosarcoma	9
Osteosarcoma	4
Chondrosarcoma	2
Ewing's sarcoma	4
Neuroblastoma	2
Lymphoma	2
Wilms' tumor	2
Ependymoma	1
Prior therapy	
Radiotherapy	0
Chemotherapy	9
Chemotherapy + radiotherapy	37
Prior chemotherapy regimens	
Median: 3.5	
Range: 1-6	

*> 21 yrs of age.
†Median age = 10 yrs, 11 mos; mean = 11 yrs, 2 mos.

ured values were nearly identical. The adult data were completely recalculated by the same programs. All parameters corresponded to those reported previously except the zero time intercepts which were found to be in error in table 1 of reference 12.

RESULTS

Characteristics of 48 patients are shown in table 1. Two patients, a 22-year-old man with Ewing's sarcoma and a 23-year-old woman with sarcoma, were excluded because of age. Several additional patients had incomplete observations and laboratory data on Days 7 and 14 following administration of the drug during at least one course of therapy. Nevertheless, all patients but two were fully evaluable for at least one course of therapy. All patients had had prior chemotherapy and most had received radiotherapy.

Hematologic Toxicity

Table 2 shows the effects of ICRF-187 on granulocyte and platelet counts and on hemoglobin concentration. Occasionally, patients and courses showed transient decreases in granulocyte and platelet counts, but there was no consistent pattern of myelosuppression with increasing dose. Hemoglobin concentration fell at all dosages tested and often required rbc transfusions. Decreases of 2-5 g/dl were seen at doses > 2250 mg/m^2/day × 3. These data contrast sharply with the adult trials where leukopenia and thrombocytopenia without anemia were seen consistently at the daily × 3 dose of > 1250 mg/m^2 (9).

Nonhematologic Toxicity

Transaminase concentrations were elevated above normal after the second or third daily dosage and usually returned to normal by 2 weeks; SGOT was always higher than SGPT (table 3). The median SGOT elevation gradually increased, with dosage reaching four and six times normal at daily × 3 doses of 3500 and 4000 mg/m^2, respectively. Alkaline phosphatase did not increase, but total bilirubin concentrations > 1.5 mg/dl were seen in one patient each at daily × 3 doses of 1000, 3250, and 3500 mg/m^2, respectively. Abnormalities in clotting tests were seen in three patients at the daily × 3 dose of 4000 mg/m^2. Prothrombin time was 15, 16.2, and 22.4 seconds (normal range, 11-12), and PTT was 52, 70, and 79 seconds (normal range, 34-37) on Days 4-6 of treatment with these patients. One patient had a decrease in fibrinogen concentration and two had decreases in specific

TABLE 2.—Hematologic toxicity in patients without evidence of marrow disease

Dose (mg/m^2/day × 3)	No. of evaluable—		Granulocyte count nadir			Platelet count nadir			Hemoglobin decrease		
	Patients	Courses	Median (× 10^3 cells/mm^3)	Range (× 10^3 cells/mm^3)	Days	Median (× 10^3 cells/mm^3)	Range (× 10^3 cells/mm^3)	Days	Median (g/dl)	Range (g/dl)	Days
1000	1	3	1.6	0.3-2.1	7-22	158	140-200	7-14	1.3	1.1-1.8	7-14
1500	1	1		1.9	3		264	21		1.6	3
1875	1	1		8.7	21		1020	21		1.4	22
2000	1	2	3.6	2.4,4.7	3-18	780	760, 870	19-20	1.1	1.0, 1.1	7-19
2250	2	3	2.9	0.9-3.1	3-22	350	280-450	11-20	2.3	1.1-2.9	22-27
2500	4	6	1.6	1.3-3.0	4-22	200	105-350	9-28	1.0	0.5-5.4	10-26
2750	2	3	3.0	2.3-5.0	14-21	170	47-226	7-21	1.3	0.6-3.8	14-22
3000	3	3	1.2	1.0-1.9	9-15	196	155-275	9-11	1.7	1.2-2.0	11-21
3250	2	2	2.5	1.7-3.3	5-11	280	188, 370	13-15	1.0	0.6, 1.3	5-11
3500	3	6	1.2	0.3*-2.2	7-13	200	59*-245	7-15	1.8	1.0-3.5*	9-21
4000	1	1		2.5	3		44	11		0.7	8

*Patient with a large pleural effusion.

factor levels. One patient was treated with fresh frozen plasma and another with vitamin K. All abnormalities resolved within 1 week. These patients had maximum SGOT elevations of 252, 606, and 1175 U/L. Thus, hepatic toxicity was considered dose-limiting and no further patients were entered at the daily × 3 dose of 4000 mg/m^2. Three additional patients were entered at the daily × 3 dose of 3500 mg/m^2. On Day 3 of treatment one of these patients had a slight increase in prothrombin time to 13.9 seconds, but no change in PTT.

For some patients, a transient elevation of serum triglyceride concentrations to a maximum of 500 mg/dl was seen in the first week with the daily × 3 doses of 500 and 1000 mg/m^2. Amylase levels were elevated in the first week in four patients with leukemia (range, 95–372 U/L). This transient change was not associated with abdominal pain and did not appear to be related to the dosage given.

A previous study had shown that the urinary clearance of iron, zinc, and calcium increased during treatment with ICRF-187 (9). In the present study, calcium concentrations were measured during 51 courses of therapy; 30 courses at all dosage levels were associated with a transient decrease in serum calcium concentrations of 0.1–1.7 mg/dl (median, 0.6). No symptoms were associated with these changes. Magnesium concentrations were unchanged. Nausea and vomiting were mild and usually did not require medication. One patient had alopecia attributable to ICRF-187.

Thus, the MTD in children appears to be 3500 mg/m^2/day × 3, based on the changes in hepatic function and coagulation abnormalities encountered when larger dosages were administered.

Pharmacokinetics

The data for each patient were fit to a two-compartment, open model with constant iv infusion by Autoan-Nonlin and by the equations used in the previously reported adult data with ICRF-187 (12). Table 4 lists the calculated parameters for six patients 9–21 years of age, and table 5 compares the means for the four patients 9–16 years of age and all six pediatric patients with the adult data. Analysis of the adult data had shown that the 30-minute, 8-hour, and 48-hour infusions gave the same pharmacokinetic parameters (12). Therefore, averages of pharmacokinetic parameters for the 30-minute and 8-hour infusions are presented in this table. The alpha- and beta-half-lives; area under the serum curves per meter squared per gram of drug administered; and microconstants, k_{12}, k_{21}, and k_{10}, were similar in children and adults. The zero time intercepts A′ and B′ were higher for children than for adults, reflecting the higher doses administered to the children.

In children 9–16 years of age, the volume of distribution in the central compartment (Vc), the total volume of distribution (Vb), and total-body clearance per kilogram were significantly larger than these values in the adults (Student's t test). When these values were normalized to body surface area rather than kilogram of body weight, they were no longer significantly different from the adult values.

These pediatric and adult data were also analyzed by a noncompartmental method (table 5). Clearance per kilogram of body weight was 1.8 times faster in children 9–16 years of age than in the adults studied. The ratio of Vss per kilogram in the 9–16-year-old children and adults is 1.6 ($P < 0.05$). The ratio decreases to 1.3 when normalized for body surface. Mean resident time was not statistically different between the age groups.

A 24-hour urine collection following the first of the three daily doses contained a mean ± SD of 60% ± 24% (median, 50%) of intact ICRF-187 in the four children ages 9–16. These values were similar to the urinary recoveries of 46% ± 10% (median, 48%) in the adult patients for the 30-minute and 8-hour infusions.

Responses

No responses were seen in the solid tumor patients. One patient with acute lymphocytic leukemia had an M_1 marrow on Day 15 but had an M_3 marrow on Day 43 despite a second course of a daily × 3 dose of 2500 mg/m^2. Four additional patients had brief clearing of lymphoblasts from the blood.

TABLE 3.—Nonhematologic toxicity

Dose (mg/m^2/day × 3)	No. of evaluable—		SGOT			SGPT		
	Patients	Courses	Median (U/L)	Range (U/L)	Days	Median (U/L)	Range (U/L)	Days
1000	3	5	121	63–336	3–7	34	33–181	3–7
1500	3	4	277	5–350	3–10	56	2–169	3–10
1875	4	6	82	50–120	2–10	38	16–76	3–12
2000	4	5	73	39–175	3–8	39	16–62	5–9
2250	5	7	74	31–139	7–13	25	2–73	3–14
2750	2	2	143	131–154	3–7	24	18–30	3–7
3000	3	3	49	39–430	4–15	48	18–97	9–15
3250	4	4	115	77–170	3–8	93	48–138	3–8
3500	6	10	160	30–340	4–24	94	5–209	3–12
4000	4	5	252	58–1175	3–8	170	23–990	3–8

TABLE 4.—Pharmacokinetic parameters of ICRF-187

Patient No.	Age (yrs)	Weight (kg)	Body surface (m^2)	Dose (mg)	α-half-life (mins)	β-half-life (mins)	2-compartment open model with iv infusion						Noncompartmental analysis		
							A' (μg/ml)	B' (μg/ml)	Vc (L/kg)	Vb (L/kg)	Clearance ml/min/kg	Clearance ml/min/m^2	MRT (mins)	Vss (L/kg)	Clearance (ml/min/kg)
1	9	25	0.89	3400	27.6	111	174	131	0.448	0.78	4.88	136	120	0.518	4.31
2	9	31	1.00	3500	7.0	95	384	70	0.249	1.15	8.36	259	95	0.795	8.39
3	15	51	1.52	5800	12.6	133	117	96	0.530	1.06	5.52	186	172	0.926	5.38
4	16	46.5	1.55	5035	16.9	113	311	80	0.277	0.86	5.27	158	112	0.588	5.25
5	20	68	1.86	5600	5.7	126	554	43	0.137	1.20	6.63	243	114	0.757	6.66
6	21	70.5	1.84	4600	7.2	125	357	69	0.153	0.72	3.97	152	124	0.423	3.42

DISCUSSION

Pediatric phase I trials of anticancer agents are necessary because the tolerance of children to these drugs may differ greatly from adults. Glaubiger et al (19) reviewed the literature on 17 anticancer drugs that had been studied in both children and adults. The ratio of MTD in children to MTD in adults varied from 0.83 to > 2 (median, 1.3). The dose-limiting toxic effect was primarily myelosuppression and was almost always identical for children and adults. A recent review of these data and new trials came to the same conclusion (20).

Several observations in this pediatric phase I study of ICRF-187 are unique. First, the MTD of 3500 mg/m^2/day × 3 is 2.8 times that of adults. Second, the dose-limiting toxic effect appears to be hepatic dysfunction, whereas it was myelosuppression in adults. Third, pharmacokinetic studies show that the volume of distribution per kilogram of the drug is significantly higher in children than in adults.

Myelosuppression was consistently seen in adult trials of ICRF-187 at three institutions using three different dosage schedules and was the major toxic effect with oral doses of ICRF-159 (1,2,9,10). In the trial using a daily × 3 dosage, the median age was 52 years. The seven patients treated at doses of 1250 and 1500 mg/m^2/day × 3 were adults. Our pediatric study enrolled a 22- and 23-year-old patient with pediatric solid tumors at 2250 and 2500 mg/m^2/day × 3, respectively. Although they were not included in the analysis because of age, both showed myelosuppression with absolute granulocyte count nadirs of 770, 520 (two courses), and 220/mm^3 and platelet count nadirs of 21,000, 25,000, and 41,000/mm^3. One of these patients had a large pleural effusion. In contrast, two patients with Ewing's sarcoma, ages 20 and 21, treated at 2500 and 3000 mg/m^2/day × 3 did not have myelosuppression. The only patients < 21 years old with a granulocyte count < 1000/mm^3 were a 12-year-old child with neuroblastoma previously treated with extensive chemotherapy and radiotherapy who had a single low count after the second of three courses at 1250 mg/m^2/day × 3 and a 15-year-old with Wilms' tumor and a pleural effusion filling two-thirds of the left chest treated with two courses at 3500 mg/m^2/day × 3. One other patient had a pleural effusion. She did not show a decrease in granulocyte count, but had a decrease in platelet count to 44,000/mm^3 after 4000 mg/m^2/day × 3. Thus, we cannot determine whether there is an abrupt change in susceptibility to myelosuppression at about age 22 or whether a pleural effusion increases the toxic effects of ICRF-187.

Decreases in hemoglobin concentration were occasionally severe and rapid (within 1–2 weeks). No sites of bleeding were found except brief hematuria in one patient during one of three courses. None of the patients was evaluated for hemolysis except by reticulocyte counts which were usually < 1%. We do not consider

TABLE 5.—Mean pharmacokinetic parameters of ICRF-187

			2-compartment open model with iv infusion						Noncompartmental analysis		
							Clearance				
	α-half-life (mins)	β-half-life (mins)	A′ (μg/ml)	B′ (μg/ml)	Vc (L/kg)	Vb (L/kg)	ml/min/kg	ml/min/m²	MRT (mins)	Vss (L/kg)	Clearance (ml/min/kg)
Pediatric data											
Mean (9–16 yrs, n = 4)	16.0	113	247	94	0.376*	0.96*	6.01*	185	125	0.707*	5.83*
SD	8.7	16	123	27	0.135	0.17	1.59	54	33	0.188	1.77
Mean (9–21 yrs, n = 6)	12.8	117	316	81	0.299	0.96*	5.77*	189	123	0.668*	5.57*
SD	8.4	14	157	30	0.159	0.20	1.54	51	26	0.189	1.76
Adult data†											
Mean (n = 5)	11.6	146	109	30	0.177	0.66	3.32	124	140	0.451	3.18
SD	2.4	28	31	5	0.038	0.18	1.32	38	27	0.101	1.33

*$P < 0.05$ relative to adult data.

†Average of data from 30-min and 8-hr infusions (12).

these changes dose-limiting. Nevertheless, rbc transfusions will probably be required during phase II trials.

Hepatic toxicity consisting of transient increases in bilirubin and transaminase levels have been seen in the adult trials of ICRF-187. These abnormalities increased in severity with dosage in our pediatric trial and became life-threatening when they were associated with abnormalities in clotting function at 4000 mg/m²/day × 3. Hepatic toxicity has been noted in preclinical studies in dogs (21). A study in dogs showed that ICRF-187 is extensively bound to tissues and is released slowly by dialysis. EDTA-containing buffers are more effective in releasing ICRF-187 than buffers without chelating agents (22). Thus, some of this difference between children and adults may be due to increased tissue binding in highly perfused organs, as it is in the liver.

The pharmacokinetic data in tables 4 and 5 suggest that some of the difference in MTD between adults and children may be explained by the clearance and volume of distribution of ICRF-187 in the two age groups. The volumes of distribution, Vc, Vb, and Vss, and clearance per kilogram of body weight in the 9–16-year-old group were 1.6 to 2.1 times the values for the adults. The increased clearance can be attributed largely to the larger volume of distribution since clearance equals Vss × β. These differences are less apparent when the data are normalized to body surface area. Doses of antitumor agents are usually normalized to body surface but physiologic fluid volumes change less with age when normalized to body weight (23,24).

There is considerable uncertainty and potential error in volume of distribution calculations, particularly with limited numbers of patients. Nevertheless, all of these calculated values show similar changes that would predict lower concentrations of ICRF-187 in children than in adults after the same dose. Additional children should be studied during the phase II evaluation of ICRF-187. It will be particularly important to determine whether these parameters change in the twenties as suggested by our observations.

These volumes of distribution and clearance differences cannot explain all of the difference in MTD, since modeling of the pharmacokinetics data shows that at the MTDs children have much higher estimated initial concentrations and areas under the plasma curve than adults. Other possible explanations for the observed improved tolerance include a decreased rate of uptake, activation, or binding of the drug or less sensitivity of critical organs like the bone marrow to the toxic effects in ICRF-187 in children. One hypothesis why children show less myelosuppression than adults is that children have a larger number of hematopoietic stem cells per unit of surface area compared to older patients (19). This hypothesis has not been tested.

Clinical responses to ICRF-187 were seen only in patients with acute lymphocytic leukemia. Usually they were minor, but one patient treated with 2250 mg/m²/day × 3 had a brief return of normal bone marrow morphology and function. There are scattered reports of bone marrow responses with oral doses of ICRF-159. Currently, the Pediatric Oncology Group is conducting a phase II study at 3000–3500 mg/m²/day × 3 to evaluate the clinical effectiveness of this dose schedule.

REFERENCES

1. BAKOWSKI MT. ICRF 159, (α)1,2-di(3,5-deoxopiperazin-l-yl)propane, NSC-129 943; razoxane. Cancer Treat Rev 3:95–107, 1976.
2. BELLET RE, ROZENCWEIG M, VON HOFF DD, ET AL. ICRF 159: current status and clinical prospects. Eur J Cancer 13:1293–1298, 1977.
3. WOODMAN RJ. Enhancement of antitumor effectiveness of ICRF-159 (NSC-129943) against early L1210 leukemia by combination with *cis*-diamminedichloroplatinum (NSC-119875) or daunomycin (NSC-82151). Cancer Chemother Rep Part 2, vol 4 (1):45–52, 1974.
4. JAMES SE, and SALSBERY AJ. Effect of (α)-1,2 bis (3,5 deoxopiperazin-l-yl) propane on tumor blood vessels and its relationship to the anti-metastatic effect in the Lewis lung carcinoma. Cancer Res 34:839–842, 1974.
5. HERMAN EH, FERRARIS VJ, JORDAN W, ET AL. Reduction of chronic daunorubicin cardiotoxicity by ICRF 187 in rabbits. Res Commun Chem Pathol Pharmacol 31:85–97, 1981.
6. WANG G, FINCH MD, TREVAN D, ET AL. Reduction of daunomycin toxicity by razoxane. Br J Cancer 43:871–877, 1981.
7. CREAVEN PJ, ALLEN LM, and ALFORD DA. The bioavailability in man of ICRF 159, a new antineoplastic agent. J Pharm Pharmacol 27:914–918, 1975.

8. REPTA AJ, BALTERZOR MJ, and BANSAL PC. Utilization of an enantiomer as a solution to a pharmaceutical problem: application to solubilization of 1,2-di:(4-piperazine-2,6-dione)propane. J Pharm Sci 65:238–242, 1976.
9. VON HOFF DD, HOWSER D, LEWIS BJ, ET AL. Phase I study of ICRF-187 using a daily for 3 days schedule. Cancer Treat Rep 65:249–252, 1981.
10. LEISMAN J, HAAS C, PETRIK B, and HOOGSTRATEN B. Phase I evaluation of ICRF 187 (NSC 169780) in patients with advanced malignancy. Proc Am Assoc Cancer Res 21:343, 1980.
11. KOELLER JM, EARHART RH, and DAVIS HL. Phase I trial of ICRF-187 by 48-hour continuous infusion. Cancer Treat Rep 65:459–463, 1981.
12. EARHART RH, TUTSCH KD, KOELLER JM, ET AL. Pharmacokinetics of (+)-1,2-di(3,5-dioxopiperazin-l-yl) propane intravenous infusions in adult cancer patients. Cancer Res 42:5255–5261, 1982.
13. EARHART RH, KAMEN BA, and HOLCENBERG J. Pharmacokinetics of (+)-1,2-di(3,5-dioxopiperazin-l-yl) propane (L-ICRF) in children. Clin Pharmacol Ther 35:238, 1984.
14. GIBALDI M, and PERRIER D. Pharmacokinetics. New York, Marcel Dekker, 1975, pp 69–77.
15. SEDMAN AJ, and WAGNER JG. AutoAn, a decision-making pharmacokinetic computer program. Ann Arbor, MI, Publication Distribution Service, 1975.
16. BROWN RD, and MANNO JE. Estrip, a basic computer program for obtaining initial polyexponential parameter estimates. J Pharm Sci 67:1687–1691, 1978.
17. PERRIER D, and MAYERSOHN M. Non-compartmental determination of the steady-state volume of distribution for any mode of administration. J Pharm Sci 71:372–373, 1982.
18. STRAUGHN AB. Model-independent steady-state volume of distribution. J Pharm Sci 71:597–598, 1982.
19. GLAUBIGER DL, VON HOFF DD, HOLCENBERG JS, ET AL. The relative tolerance of children and adults to anticancer drugs. Front Radiat Ther Oncol 16:42–49, 1981.
20. MARSONI S, UNGERLEIDER RS, HURSON SB, ET AL. Tolerance to antineoplastic agents in children and adults. Cancer Treat Rep 69:1263–1269, 1985.
21. LEVINE BS, HENRY MC, PORT CD, ET AL. Preclinical toxicologic evaluation of ICRF-187 in dogs. Cancer Treat Rep 64:1211–1215, 1980.
22. MHATRE RM, TEW KD, VANHENNIK MB, ET AL. Absorption, distribution and pharmacokinetics of ICRF 187 in dogs. Proc Am Assoc Cancer Res 24:290, 1983.
23. FRIIS-HANSEN B. Body water compartments in children: changes during growth and related changes in body composition. Pediatrics 28:169–181, 1961.
24. SIEGEL SE, and MORAN RG. Problems in the chemotherapy of cancer in the neonate. Am J Pediatr Hematol Oncol 3:287–296, 1981.

Comparison of Alternating to Nonalternating Chemotherapy Regimens for Non-Small Cell Lung Cancer[1,2]

Thomas J. Meyer,* Nathan W. Pearlman, Paul E. Siebert, James R. Murphy, Madeline J. White, Arlene Zaloznik, Thomas Braun, and Patricia Rempel[3,4]

Forty-seven patients with stage III lung cancer from four institutions in the Denver area were entered in a study comparing two regimens of chemotherapy. The patients were randomized into two groups: Group A received lomustine, cyclophosphamide, vincristine, cisplatin, and doxorubicin monthly; Group B received the five-drug regimen on Months 1, 3, and 5 and received 5-FU by constant infusion, methotrexate, and mitomycin on Months 2, 4, and 6. The age, extent of disease, Karnofsky score, prior therapy, and average number of chemotherapy courses received in each group were comparable. The median survival in Group A was 265 days and in Group B was 163 days ($P > 0.25$). There does not seem to be an advantage in survival in patients who are treated with the eight-drug regimen over the five-drug regimen. [Cancer Treat Rep 70:711–714, 1986]

In 1983, we reported a 47% response rate in non-small cell lung cancer using a five-drug combination of cyclophosphamide, vincristine, lomustine, cisplatin, and doxorubicin (1). Earlier, Miller et al (2) published their results using a three-drug regimen consisting of 5-FU, vincristine, and mitomycin, with a similar response rate. Since two of the three drugs in their regimen were different from ours, and methotrexate, a drug shown to be active in other studies, was not present in either regimen, we decided to study an alternating regimen which would involve eight drugs in an attempt to improve both response and survival rates in inoperable stage III non-small cell lung cancer. Evidence in small cell carcinoma for synergistic activity with such alternating regimens had been published (3). Since that time, however, other studies have suggested that more intensive alternating treatment in small cell cancer has not been helpful (4–6). Our study suggests that the alternating regimen described here is no better than the five-drug regimen given alone when response rate or survival is compared in the two groups.

PATIENTS AND METHODS

Seventy-five patients from four institutions in the Denver area (Veterans Administration Medical Center, University of Colorado Health Sciences Center, Fitzsimons Army Medical Center, and Denver General Hospital) were entered in the study. Patients were eligible for inclusion if they had not received chemotherapy or radiation therapy in the past month, had measurable or evaluable disease, had histologic proof of epithelial lung cancer, could give informed consent, and were willing to enter the hospital at least monthly for therapy. Since we wanted to see the practicability of treating every patient with disseminated lung cancer seen in our institutions, we did not exclude patients with a short life expectancy or a very low Karnofsky score. Before beginning the study, we calculated that 100 patients in each group would be required to show an improvement in response rate from 47% [the results from our previous study (1)] to 80%. This was based on a one-tailed test of difference in proportion of response at the 0.05 level of significance. We chose to close the study after entering only 75 patients because the analysis done at that point, and subsequently discussed, indicated that it was highly unlikely that significant difference would ever exist between the groups. Patients with disease limited to one hemithorax were considered to have limited disease.

[1]Received Apr 25, 1985; revised Dec 12, 1985; accepted Dec 13, 1985.

[2]The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

[3]Departments of Medicine, Surgery, Radiology, and Nursing, Veterans Administration Medical Center; Department of Bio-Statistics, University of Colorado Health Sciences Center; Department of Medicine, Denver General Hospital; and Department of Medicine, Fitzsimons Army Medical Center, Denver, CO.

[4]We thank Anna Barón, PhD, Department of Preventive Medicine and Biometrics, University of Colorado Health Sciences Center, for statistical assistance.

*_Reprint requests to:_ Dr. Thomas J. Meyer, Denver Veterans Administration Medical Center, 1055 Clermont St, Denver, CO 80220.

However, since a concurrent protocol was studying patients who had very limited disease, all of the patients in this study had multicentric or massive disease which was too large to be encompassed in a standard radiation field. Patients with extensive disease had disease outside one hemithorax. They were randomized by means of a random number table into two groups as follows. Group A consisted of lomustine (50 mg/m^2 orally), cyclophosphamide (300 mg/m^2 iv), vincristine (1.5 mg/m^2 iv), cisplatin (50 mg/m^2 iv), and doxorubicin (50 mg/m^2 iv). All chemotherapy was given on Day 1 of each cycle and repeated monthly. The regimen was given on Months 1, 3, and 5. Group B consisted of 5-FU (20 mg/kg/day on Days 1 and 2 by constant infusion over 48 hours; 500 mg twice a day orally on Days 3 and 4), methotrexate (30 mg/m^2 by iv push on Day 1), and mitomycin (10 mg/m^2 by iv push on Day 1). The regimen was given on Months 2, 4, and 6.

The chemotherapy was given for a total of six courses or until disease progression, patient refusal, or loss to follow-up. The five-drug regimen was adjusted to achieve total wbc count nadirs of 2000-3000/mm^3 at 14 days after chemotherapy. It proved impractical to adjust the wbc count after the 5-FU, methotrexate, and mitomycin, and consequently, standard doses were given to all patients.

For the purposes of this analysis, 47 patients who received at least two courses of chemotherapy were included. Twenty-eight patients were dropped from analysis because they did not receive at least two courses of chemotherapy. The reasons for dropping these patients are shown in table 1. Response to chemotherapy was determined by a radiologist (P. E. Siebert), who was blinded to the time sequence of the comparison films. Films taken before chemotherapy were coded and compared to coded films taken 3-6 months after initiation of chemotherapy. Comparison was made using standard measurements of the product of the diameters or by Eagan's method of evaluable disease as described previously (1). Survival curves were constructed according to the Kaplan-Meier procedure and comparison of survival was done by the log-rank method (7). Survival was measured from the first day of chemotherapy until the day of death or until withdrawal of patients from the study. The final data were organized and collated by Thomas J. Meyer and reviewed by the other investigators.

RESULTS

Table 2 shows the patient characteristics in both the five- and eight-drug alternating groups. All patients were male except one female in each group. There were more large cell carcinomas in the eight-drug group and more adenocarcinomas in the five-drug group. The disease extent was similar in both groups but there were more patients in the eight-drug group who had had prior radiation therapy. No patient in either group had received previous chemotherapy. Thirty-five percent of the eight-drug group and 20% of the five-drug group had been previously irradiated. The average number of courses of chemotherapy given was similar in each group. Although a full course of treatment would have been six courses of chemotherapy, only eight patients received a full six courses. Most of the patients died or failed in fewer than those six courses (table 2). Fifteen patients received two courses, ten received three courses, four received four courses, and six received five courses. Thirty percent responded in the five-drug group and 33% responded in the eight-drug group. There were two complete responders in Group A and three complete responders in Group B. Figure 1 shows the survival curves from the two chemotherapy groups. There was no significant difference in median survival between Group A (265 days) and Group B (163 days; $P > 0.25$). The possibility of a Type II error for detecting a doubling of survival is $< 20\%$ (7). The 25th to 75th quartile ranges for the survival times are 158-585 days for Group A and 121-264 days for Group B. An approximate 90% two-sided confidence limit for the difference in response rates ranges from 19% favoring the five-drug combination to 25% favoring the eight-drug combination. This is consistent with a substantial difference in response rates, although not statistically significant. The number of patients analyzed is too small to draw reliable

TABLE 1.—Reasons for dropping patients from study*

Reason	Group A	Group B
Lost after 1 course	3	7
Dead after 1 course	10	6
Refused after 1 course	2	0

* Values = No. of patients.

TABLE 2.—Patient characteristics*

Characteristic	Group A	Group B
Total	23	24
Age in yrs (range)	61 (43-77)	62 (49-74)
Karnofsky score (range)	71% (50%-90%)	67% (30%-90%)
Histology		
Adenocarcinoma	12	7
Squamous cell carcinoma	8	8
Large cell carcinoma	3	9
Extent of disease		
Limited	33%	33%
Extensive	67%	67%
Prior radiation therapy	20%	35%
Average No. of chemotherapy courses	3.5	3.7
Responders	30%	33%

* Unless otherwise specified, values = No. of patients.

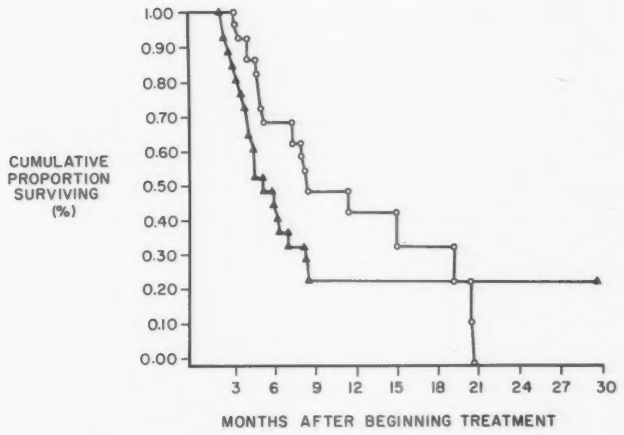


FIGURE 1.—Cumulative proportion of patients surviving after initiation of chemotherapy. O = 5-drug combination (Group A); ▲ = 8-drug alternating regimen (Group B).

conclusions about relative response rates to the two treatments. The 90% confidence interval for the ratio of median survival times of the eight-drug group versus the five-drug group ranges from 0.82 to 2.52.

DISCUSSION

Response rates and survival in the two arms of this study appear to be similar. Although systematic analysis of toxicity was not performed, patients receiving the alternating eight-drug regimen seemed to have less toxicity due to a decreased incidence of nausea, vomiting, and malaise after receiving the regimen not containing cisplatin. Conversely, the 5-FU infusion required 2 days of hospitalization, whereas the five-drug combination could be given to outpatients. Thus, each approach had relative advantages and disadvantages.

The 30% response rate seen with the five-drug combination in this study is less than the 47% we reported earlier; however, survival at 1 year is the same in both studies (37% vs 34%) (1). The patient population in the two studies was different, however. In the earlier study, all patients with stage III lung cancer were included. In the present study, patients with very limited stage III disease were entered in a separate protocol which was designed to study irradiation alone versus irradiation plus chemotherapy. Takita et al (8) reported similar findings when comparing early experience with this combination to that with a later modified regimen. It appears that greater response rates in non-small cell lung cancer do not automatically result in longer survival (9,10). Our disappointing experience with alternating chemotherapy regimens mirrors that of others in small cell carcinoma (4-6), who were unable to repeat the success with this technique suggested earlier (3). Finally, our inability to improve response by adding more drugs to a platinum-based regimen is similar to the experience of Kelsen et al (11) and Itri et al (12), who found no benefit from the addition of either cyclophosphamide, cyclophosphamide plus doxorubicin, or bleomycin to the combination of vindesine and cisplatin.

Our results indicate that the eight-drug alternating regimen described here offers little promise for improved control of disseminated non-small cell lung cancer.

REFERENCES

1. PEARLMAN NW, MEYER TJ, SIEBERT PE, ET AL. Cyclophosphamide, vincristine, lomustine, cisplatin, and doxorubicin in the treatment of non-small cell lung cancer. Cancer Treat Rep 67:375-376, 1983.
2. MILLER TP, MCMAHON LJ, and LIVINGSTON RB. Extensive adenocarcinoma and large cell undifferentiated carcinoma of the lung treated with 5-FU, vincristine, and mitomycin-C (FOMi). Cancer Treat Rep 64:1241-1245, 1980.
3. COHEN MH, IHDE DC, BUNN PA, JR, ET AL. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 63:163-170, 1979.
4. AISNER J, WHITACRE M, VAN ECHO DA, ET AL. Combination chemotherapy for small cell carcinoma of the lung: continuous versus alternating non-cross-resistant combinations. Cancer Treat Rep 66:221-230, 1982.
5. MARKMAN M, PENDERGRASS KB, and ABELOFF MD. Intensive timed sequential combination chemotherapy in extensive-stage small cell carcinoma of the lung. Cancer Treat Rep 66:1880, 1982.
6. LININGER TR, FLEMING TR, and EAGAN RT. Evolution of alternating chemotherapy and sites and extent of disease in extensive small cell lung cancer. Cancer 48:2147-2153, 1981.
7. LEE ET. Statistical methods for survival data analysis. Belmont, CA, Wadsworth Publishers, 1980, p 557.
8. TAKITA H, EDGERTON F, MORABELLA P, ET AL. Platinum-based

combination chemotherapy in non-small cell lung cancer. Cancer 48:1528-1530, 1981.
9. WEISS GB, BUNCE H, and HOKANSON JA. Comparing survival of responders and non-responders after treatment: a potential source of confusion in interpreting cancer clinical trials. Controlled Clin Trials 4:43-52, 1983.
10. OYE RK, and SHAPIRO MF. Reporting results from chemotherapy trials. Does response make a difference in patient survival? JAMA 252:2722-2725, 1984.
11. KELSEN D, GRALLA R, STOOPLER M, ET AL. Cisplatin, doxorubicin, cyclophosphamide, and vindesine combination chemotherapy for non-small cell lung cancer. Cancer Treat Rep 66:247-251, 1982.
12. ITRI LM, GRALLA RJ, KELSEN DP, ET AL. Cisplatin, vindesine and bleomycin (CVB) combination chemotherapy of advanced non-small cell lung cancer. Cancer 51:1050-1055, 1983.

Relationship of Glutathione Depletion and Inhibition of Glutathione-*S*-Transferase Activity to the Antimitotic Properties of Estramustine[1,2]

Kenneth D. Tew,[3,4,*] Alesia Woodworth,[3] and Mark E. Stearns[3,4]

A depletion of intracellular glutathione (GSH) is accompanied by a subsequent inhibition of GSH-*S*-transferase activity in a DU145 human prostatic carcinoma cell line treated with cytotoxic concentrations of estramustine. When GSH depletion reached approximately 50% of normal (approximately 2 hours after 10 μM estramustine), the mitotic index of logarithmically dividing cultures began to increase. A linear increase from 3.14% to 22.5% occurred during the period of 2–24 hours following 10 μM estramustine. Morphological studies showed that mitotic figures accumulated in metaphase and had abnormalities consistent with spindle malformation. Nocodazole and cytochalasin B also possess anticytoskeletal properties, but had little effect upon the intracellular levels of GSH or its associated transferase enzymes. The constituent moieties of estramustine, estradiol, and nor-mechlorethamine had effects on thiol metabolism which were dissimilar from estramustine, confirming previous findings that the unmetabolized parent drug is responsible for pharmacological activity. Estramustine had no effect upon GSH reductase activity, suggesting that drug toxicity was not a general thiol phenomenon. Buthionine sulfoximine decreased intracellular GSH in DU145 cells and enhanced the cytotoxic potential of estramustine in this cell line. The anticytoskeletal and antimitotic properties of estramustine may be enhanced by the drug-induced GSH imbalance and subsequent effects on GSH-*S*-transferase enzymes.
[Cancer Treat Rep 70:715–720, 1986]

The cytotoxic mechanism of action of estramustine is well documented as being independent of its constituent steroid/mechlorethamine moieties (1-3) and more recently has been shown to reside in its antimicrotubule effects (4-6). The precise nature of the reported antimitotic and stathmokinetic effects of the drug are not characterized, although there is some preliminary evidence that microtubule-associated proteins may be principal targets (6,7). Many proteins contribute to the nonstochastic organization of the cytoskeleton, and in dividing cells these elements reorganize as the cell enters mitosis. Previous studies have suggested that thiol-containing macromolecules have a role in the organization of the mitotic spindle during cell cycle transition (8-10). In addition, the vinca alkaloids have been shown to cause a shift in the thiol:disulfide ratio in some cell lines with concomitant effects on protein synthesis (11). Both glutathione (GSH) and the series of GSH-*S*-transferase isoenzymes are critical to sulfhydryl balance within cells and they also serve to detoxify a wide range of xenobiotic compounds. Because thiols and their related enzymes are so critical to cell survival and cell cycle progression, this study has considered the effects of estramustine on both GSH levels and GSH-*S*-transferase activity in a human prostate tumor cell line and relates these effects to the observed antimitotic activities of the drug. DU145 cells were studied since estramustine is employed primarily in the treatment of prostatic cancer and the drug is known to concentrate in these cells (4).

MATERIALS AND METHODS

Cell Culture

DU145 human prostatic carcinoma cells were grown as monolayer cultures in RPMI 1640 (Advanced Biotechnologies, Silver Spring, MD) supplemented with 10% fetal calf serum (K. C. Biologicals, Lenexa, KS), 4 mM glu-

[1]Received June 11, 1985; revised Nov 27, 1985; accepted Dec 23, 1985.

[2]Supported in part by Public Health Service grant CA-28806 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from the A. B. Leo Helsingborg Research Foundation.

[3]Division of Medical Oncology (*K. D. Tew* and *A. Woodworth*), Department of Pharmacology (*K. D. Tew*), and Department of Anatomy (*M. E. Stearns*), Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC.

[4]*Present address:* Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA.

**Reprint requests to:* Kenneth D. Tew, PhD, Department of Pharmacology, Fox Chase Cancer Center, 7701 Burholme Ave, Philadelphia, PA 19111.

tamine, and penicillin/streptomycin at 37°C under an atmosphere of 95% air, 5% carbon dioxide. For collection of samples, cells were detached by incubating with 0.08 M sodium citrate for 2 mins at 37°C.

For mitotic index (MI) determinations and other mitosis studies, cells were grown directly on sterilized glass slides (Thomas Scientific, Philadelphia, PA) and placed in 100 × 15-mm plastic petri dishes (Becton Dickinson, Oxnard, CA). When attached cells had formed discrete colonies of eight to 32 cells, the cultures were considered to be in logarithmic growth and were used for drug experiments.

Mitotic Studies

Slides were removed from the petri dishes and placed in a bath of 3:1 methanol:acetic acid containing 4 mM $MgCl_2$ and 1.5 mM $CaCl_2$. After 15 mins these slides were transferred to fresh fixative and this was followed by a third fixation. After air drying, slides were placed in 7% perchloric acid at 4°C for 24 hours. Slides were exhaustively washed in deionized water and stained in a filtered solution of 0.5% brilliant blue R (Sigma, St. Louis, MO) and 0.5% safranin O (Sigma) in 10% acetic acid. Slides were viewed after 2-3 hours to judge stain intensity. Generally, a 4-hour period produced a differential spindle/chromosome/cytoplasm stain. Scoring of cells in prophase, metaphase, anaphase, telophase, or interphase was as previously described (10,12). MI values were calculated from the formula

$$\frac{(P+M+A+T)}{(P+M+A+T)+(I)} \times 100.$$

Thiol Measurements

Assays for total cellular GSH (13), GSH reductase (14), and GSH-S-transferase (15) have been described previously.

Cell Survival Studies

Measurement of the colony-forming potential of the DU145 cells has been described previously (4). Colonies were scored using a Biotran III Automatic Totalizer (New Brunswick, NJ).

Drugs

Estramustine was obtained from A. B. Leo, Inc (Helsingborg, Sweden). Nor-mechlorethamine (nor-HN2) and nocodazole were obtained from Dr. Ven Narayanan (Drug Development Branch, National Cancer Institute, Bethesda, MD). Estradiol, cytochalasin B, and buthionine sulfoximine (BSO) were purchased from Sigma. Nocodazole was dissolved in DMSO, BSO in media, and the others in absolute ethanol.

RESULTS

Using a colony-forming assay, 10 μM estramustine was found to be an approximate ID_{30}-ID_{40} concentration in DU145 cells. Figure 1 shows the effects of this drug concentration on MI (*see* insert) and three aspects of GSH metabolism. In the absence of drug, these cells had a normal MI of 3.14, with slightly greater than half of the mitotic figures being metaphase (table 1). With increasing time of incubation there was a drug-induced accumulation of cells in metaphase, which was reflected by the substantial increase in MI. In addition, by 4 hours following estramustine, > 95% of the metaphase figures were abnormal, with the most prevalent alterations being scattered chromosome figures and inadequately stained spindles. At higher drug concentrations (table 1), the MI dropped and there was a high level of cytolysis in interphase cells. Total cellular GSH dropped to 60% of normal within 1 hour of drug exposure and remained low through the 24-hour period. GSH-S-transferase activity also dropped to approximately 40% of normal at 24 hours

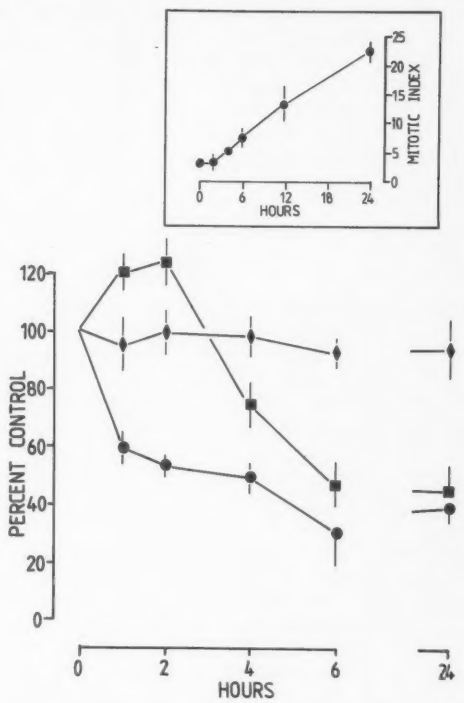


FIGURE 1.—Effect of 10 μM estramustine on MI and aspects of GSH metabolism in DU145 cells. Log-phase cells were harvested and assayed for GSH reductase (◆). ■ = GSH-S-transferase; ● = total cellular GSH. MI (insert) was determined as percentage of stained cells scored as prophase, metaphase, anaphase, or telophase. Data are mean ± SD of at least 3 experiments.

TABLE 1.—Effect of estramustine on the mitotic phase of DU145 cells*

Concentration of estramustine (μM)	Incubation time (hrs)	% of cells				
		P	M	A	T	MI
0	0	17.0	53.6	9.8	19.6	3.14
10	2	9.5	89.3	0.6	0.6	3.67
10	4	2.8	97.2	0	0	5.55
10	6	2.8	97.2	0	0	7.72
10	12	1.8	98.2	0	0	13.4
10	24	0.6	99.4	0	0	22.5
5	24	0.6	99.4	0	0	26.3
20	24	1.7	98.3	0	0	9.21
25	24	0	100	0	0	3.92

*DU145 cells were attached to slides and treated as described in the Materials and Methods section. At least 3000 cells were scored from duplicate experiments to calculate MI. Calculation of mitotic phase (P= prophase, M = metaphase, A = anaphase, and T = telophase) was based on the scoring of at least 600 mitotic figures for control and at least 300 mitotic figures for drug-treated cells.

after an initial increase in activity in the first 2 hours following drug exposure. GSH reductase activity was not affected by estramustine treatment.

A time course of the effects of estradiol or nor-HN2 on GSH or GSH-*S*-transferase activity is shown in figure 2. Estradiol at 100 μM had little effect on these values, while nor-HN2, as might be expected for an electrophile-producing drug, caused a significant short-term depletion of GSH and an approximate 30% inhibition of GSH-*S*-transferase activity at 4 hours following exposure to the drug. By 24 hours, both GSH and transferase activity were back to control levels.

Using 24-hour incubations, comparisons of equitoxic concentrations of estramustine, estradiol, and nor-HN2 on GSH (fig 3) and GSH-*S*-transferase activity (fig 4) were made. Equivalent ID values were estimated from colony-forming assays and represent a scale of increasing drug concentrations where zero was control and ID_{100} a lethal concentration for this incubation period. Estramustine depleted intracellular GSH in a concentration-dependent manner (where the ID_{90} concentration was 25 μM). Estradiol also depleted GSH, but the ID_{50} concentration corresponded to 500 μM. In agreement with the data in figure 2, nor-HN2 caused some increase in GSH at low concentrations with little effect at higher drug concentrations (ID_{95} = 10 μM). It should be noted that short-term depletions (cf fig 2) would not be reflected in these 24-hour studies.

Similar approaches showed that only estramustine caused a depletion in GSH-*S*-transferase activity (fig 4). Both estradiol and nor-HN2 treatments caused little change in enzyme activity, although nor-HN2 caused a consistent slight increase in activity over control.

To determine if the effects of estramustine were a reflection of its general anticytoskeletal properties, similar studies were carried out using nocodazole and cytochalasin B. Figure 5 shows that pharmacologically active concentrations of each compound had little influence on the GSH levels or GSH-*S*-transferase activities in DU145 cells. The latter were slightly diminished; however, no dose-response relationship was apparent. Time course experiments with nocodazole or cytochalasin B (similar to those shown in figs 1 and 2) demonstrated a similar lack of effect on either GSH or GSH-*S*-transferase activity (data not shown).

A series of experiments were carried out to determine the effect of prior GSH depletion on subsequent estramustine exposure. Figure 6 shows that BSO pretreatment reduced the surviving fraction of DU145 cells ex-

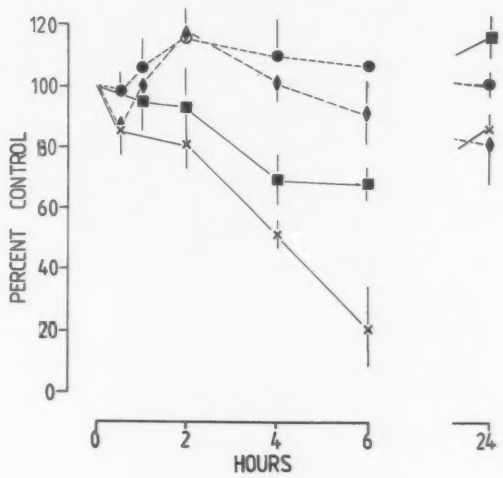


FIGURE 2.—Effect of nor-HN2 and estradiol on GSH and GSH-*S*-transferase activity in DU145 cells. Log-phase cells were exposed to 100 μM estradiol (◆ = GSH; ● = GSH-*S*-transferase) or 20 μM nor-HN2 (X = GSH; ■ = GSH-*S*-transferase). Data are mean ± SD of at least 3 experiments. Control GSH values = 8.4×10^{-7} mols/mg of protein. Control GSH-*S*-transferase activity = 36.7 nmols/min/mg of protein.

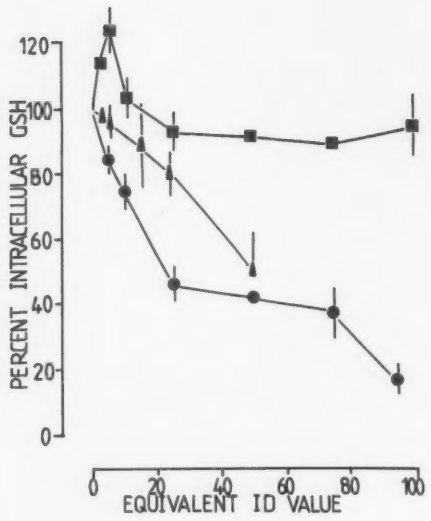


FIGURE 3.—Comparison of equitoxic concentrations of estramustine (●), estradiol (▲), and nor-HN2 (■) on intracellular GSH levels in DU145 cells. Log-phase cells were exposed for 24 hrs to concentrations of each drug which had been previously determined to cause specific growth inhibition (ie, ID_{50} would be that drug concentration causing 50% growth inhibition). Data are mean ± SD of at least 3 experiments.

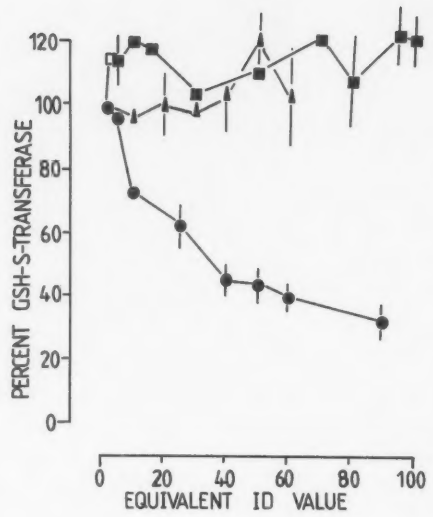


FIGURE 4.—Comparison of equitoxic concentrations of estramustine (●), estradiol (▲), and nor-HN2 (■) on GSH-*S*-transferase activity in DU145 cells. Details of the experiments are as for fig 3.

posed to subsequent estramustine. BSO alone reduced the plating efficiency from 60% to 40%; however, the BSO/estramustine combination data were calculated using the lower value as control. Thus, the actual surviving fraction following the drug combination was comparatively lower. Intracellular GSH levels were found to be approximately 6% of normal (9×10^{-7} mols GSH/mg of protein) following the two 50-μmol/L BSO treatments. By using a fluorescent derivative of estramustine (4), we were able to show that the drug entered the BSO-treated DU145 cells at a faster initial rate than the controls. BSO had no effect alone on the GSH-*S*-transferase activities, which remained between 30 and 40 nmols/min/mg of protein.

DISCUSSION

Because the carbamate ester linkage of estramustine undergoes minimal cleavage in vitro (16), there is no release of electrophilic alkylating species (1-3). Therefore, the observed dose-dependent depletion of the nucleophilic scavenging GSH and its associated transferase enzymes must be a consequence of either a direct estramustine effect or an indirect result of the anticytoskeletal effects of the drug. The efficacy of estramustine in prostate cells is enhanced by the estramustine-binding protein which serves to concentrate the drug in these cells (17). The antimitotic properties of the drug in dividing cells are reported as the drug's principal antitumor activity, although nondividing cells which lack the estramustine-binding protein are still susceptible to the anticytoskeletal effects at high drug concentrations (6). Nocodazole and cytochalasin B represent drugs which produce cytotoxic effects through disruption of cytoskeletal elements, but are considered to have intracellular targets which are different from those of estramustine (18). Since these compounds caused minimal alterations in either GSH levels or GSH-*S*-transferase activity, it is not likely that the thiol depletions caused by estramustine are merely an indirect result of the breakdown of cytoskeletal integrity. Using in vitro studies, with either a purified rat liver GSH-*S*-transferase preparation or a commercial transferase preparation (Sigma), we have shown that estramustine per se does not inhibit GSH-*S*-transferase activity even after a 24-hour incubation at 30 μM (unpublished observation). In addition, GSH reductase activity remained unaltered following estramustine treatment, suggesting that a general drug effect on GSH-related enzymes did not occur.

In other studies where GSH levels have been reduced, there is evidence that cells from diverse sources such as sea urchins (8), V-79 Chinese hamster cells (9), or Walker 256 breast carcinoma cells (10) cannot complete mitosis. Problems related to spindle formation appear to prevent the successful transition of cells through mitosis (10). Since steroids and related molecules are known to bind hydrophobically to GSH, a reaction catalyzed by GSH-*S*-transferases (19), it is possible that this and the subsequent inhibition of GSH-*S*-transferase activity may be a

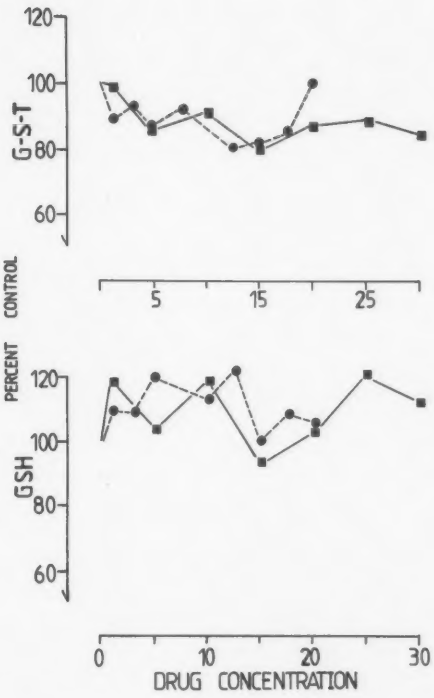


FIGURE 5.—Effects of nocodazole (——■——) and cytochalasin B (---●---) on GSH-*S*-transferase activity (top panel) and intracellular GSH (bottom panel) in DU145 cells. Log-phase cells were exposed to each drug for 24 hrs at the micromolar concentrations indicated. Results are means of duplicate experiments.

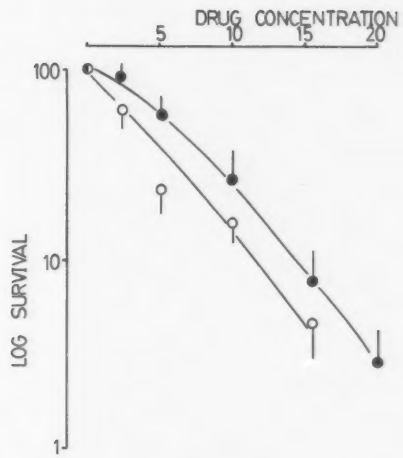


FIGURE 6.—Influence of BSO pretreatment of DU145 cells on the cytotoxic effects of estramustine. Fifty micromolar BSO was added 22 hrs prior to and at the same time as (total, 100 μmols) the micromolar concentrations of estramustine. This was followed by a further 24-hr incubation. At this time the drug-containing media was removed and replaced by fresh media. Colony fixation and staining have been described previously (ref 4). ● = estramustine only; ○ = BSO pretreatment followed by estramustine. Data are mean ± SD of 3 experiments. Normal GSH levels in untreated cells were 9.02×10^{-7} mols/mg of protein. This value was reduced to 6.25×10^{-9} mols/mg of protein by the BSO treatment.

factor in contributing to the reported anticytoskeletal and antimitotic properties of the drug. Further analysis of the precise isoenzyme profile of the DU145 cells is ongoing and it is possible that a specific GSH-*S*-transferase is involved in recognizing estramustine. A build-up of cells in metaphase and an increase in MI begins to occur 2 hours after exposure to estramustine and is linear thereafter. At this time, intracellular GSH levels are reduced to approximately 50% of normal and remain depressed throughout the drug incubation period. From such data it could be concluded that passage of cells through mitosis requires normal supplies of GSH and perhaps, as suggested for the vinca alkaloids (11) and various thiol-depleting agents (9), a correct thiol:disulfide balance. Precise estimates of threshold concentrations of intracellular GSH are of limited value in predicting drug effects in these cultured cells. In asynchronous log-phase cells there will be a great deal of heterogeneity with respect to intracellular GSH levels. In addition, a marked degree of intracellular compartmentalization of GSH is known to exist (20) and this could be relevant to the site of action of estramustine. Notwithstanding these issues, it is interesting to note that BSO, an agent known to deplete intracellular GSH (21), increased the cytotoxic potential of estramustine. As a single agent, BSO reduced the plating efficiency of the DU145 cells, suggesting that depletion of GSH per se had toxic consequences in this cell line.

It is also interesting that microtubule-associated ATPase is influenced by the cellular thiol status and by sulfhydryl inhibitors (22). Furthermore, observations of dynein ATPase activity in sperm tail indicate that estramustine has an inhibitory effect upon this enzyme (23). The present data showing thiol depletion by estramustine suggest that the drug activity might result in a block in one or both of the two types of active sulfhydryl groups found in ATPases (24).

Comparative studies of the effect of nor-HN2 and estradiol were carried out, even though there is no evidence for their presence following in vitro estramustine treatment (16). The results suggested that the electrophilic mustard species produced by nor-HN2 caused a transient suppression of intracellular GSH and transferase activity, both of which had returned to normal following 24 hours of drug exposure. Estradiol, even at 100 μM (approximately 20 times the concentration expected if estramustine were susceptible to cleavage of the carbamate bond), had little effect on either aspect of GSH metabo-

lism, although at high (nonphysiological) concentrations the steroid caused a depletion in intracellular GSH (cf fig 3). Nevertheless, the observed effects of estramustine were, in agreement with previous reports (1-3), distinct and dissimilar from its constituent chemical moieties.

REFERENCES

1. TEW KD, ERICKSON LC, WHITE G, ET AL. Cytotoxicity of a steroid nitrogen mustard derivative through non-DNA targets. Mol Pharmacol 24:324-328, 1983.
2. TEW KD, and HARTLEY-ASP B. Cytotoxic properties of estramustine are unrelated to its alkylating and steroid constituents. Urology 23:28-33, 1984.
3. TEW KD. The mechanism of action of estramustine. Semin Oncol 10:21-26, 1983.
4. STEARNS ME, JENKINS DP, and TEW KD. Dansylated estramustine, a novel probe for studies of estramustine uptake and identification of intracellular targets. Proc Natl Acad Sci USA 82:8483-8487, 1985.
5. KANJE M, DEINUM J, WALLIN M, ET AL. Effect of estramustine phosphate on the assembly of isolated bovine brain microtubules and fast axonal transport in the frog sciatic nerve. Cancer Res 45:2234-2239, 1985.
6. STEARNS ME, and TEW KD. Antimicrotubule effects of estramustine, an antiprostatic tumor drug. Cancer Res 45:3894-3897, 1985.
7. WALLIN M, DEINUM J, and FRIDEN B. Interaction of estramustine phosphate with microtubule associated proteins. FEBS Lett 179:289-293, 1985.
8. KIMURA I. Further evidence of the similarity of microtubule protein from mitotic apparatus and sperm tail of the sea urchin, as a substrate in thiol:disulfide exchange reaction. Exp Cell Res 79:445-448, 1973.
9. ONFELT A. Spindle disturbances in mammalian cells. I. Changes in the quantity of free sulfhydryl groups in relation to survival and C-mitosis in V-79 Chinese hamster cells after treatment with colcemid, idamide, carbaryl and methyl mercury. Chem Biol Interact 46:201-207, 1983.
10. TEW KD, KYLE G, JOHNSON A, ET AL. Carbamoylation of glutathione reductase and changes in cellular and chromosome morphology in a rat cell line resistant to nitrogen mustards but collaterally sensitive to nitrosoureas. Cancer Res 45:2326-2333, 1985.
11. BECK WT. Increase by vinblastine of oxidized glutathione in cultured mammalian cells. Biochem Pharmacol 29:2333-2337, 1980.
12. WISSINGER WL, ESTERING DN, and WANG RJ. A differential staining technique for simultaneous visualization of mitotic spindle and chromosomes in mammalian cells. Stain Technol 56:221-226, 1981.
13. GRIFFITH OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 106:207-214, 1980.
14. MIZE CE, and LANGDAU RG. Hepatic glutathione reductase. I. Purification and general kinetic properties. J Biol Chem 237:1589-1595, 1962.
15. HABIG WH, PABST MJ, and JACKOBY WB. Glutathione-S-transferase A: the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130-7139, 1974.
16. HARTLEY-ASP B, and GUNNARSSON PO. Growth and cell survival following treatment with estramustine, nor-nitrogen mustard, estradiol and testosterone of a human prostatic cancer cell line (DU145). J Urol 127:818-822, 1982.
17. FORSGREN B, BJORK P, CARLSTROM K, ET AL. The presence in rat and human prostate of proteins that bind steroid cytostatic complexes. Prog Clin Biol Res 75A:391-407, 1981.
18. HOEBEKE J, VAN NIJEN G, and DEBRABENDER M. Interaction of nocodazole, a new antitumoral drug, with rat brain tubulin. Biochem Biophys Res Commun 69:319-324, 1976.
19. MEISTER A, and ANDERSON ME. Glutathione. Annu Rev Biochem 52:711-760, 1983.
20. GRIFFITH OW, and MEISTER A. Origin and turnover of mitochondrial glutathione. Proc Natl Acad Sci USA 82:4668-4672, 1985.
21. GRIFFITH OW, and MEISTER A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). J Biol Chem 254:7558-7560, 1979.
22. WALLIN M, LARSON H, and EDSTROM A. Effects of sulfhydryl reagents on brain microtubule-associated ATPase activity in vitro. J Neurochem 33:1095-1099, 1979.
23. STEARNS ME, and TEW KD. The antiprostatic tumor drug estramustine binds microtubule associated proteins in vivo and in vitro. Cancer Res, 1986. In press.
24. SEKINE T, and KIELLY WW. The enzymatic properties of *N*-ethylmaleimide modified myosin. Biochim Biophys Acta 81:336-345, 1964.

Phase I Trial of Menogaril Administered as an Intermittent Daily Infusion for 5 Days[1,2]

Lawrence M. Sigman, David A. Van Echo,* Merrill J. Egorin, Margaret Y. Whitacre, and Joseph Aisner[3]

Menogaril, a semisynthetic derivative of nogalomycin, was brought to phase I clinical testing in patients with refractory solid tumors. Twenty-seven patients received 50 evaluable courses. Menogaril was given as a 1–2-hour iv infusion on 5 consecutive days, with courses repeated every 4 weeks, provided there was reversal of all drug-related toxic effects. The starting dose was 3.5 mg/m²/day × 5, with escalations in subsequent cohorts of patients to 56 mg/m²/day × 5. Neutropenia was dose dependent and dose limiting. At 56 mg/m²/day × 5, the median wbc count nadir was 1100/μl, and two of four patients were hospitalized for fever and suspected bacteremia. At 50 mg/m²/day × 5, the wbc count nadir was 2300/μl. Platelet toxicity was less severe. Nonhematologic toxicity consisted primarily of local urticaria and moderate to severe phlebitis at the infusion site, which were dose dependent and lasted up to 6 weeks. For phase II studies, the recommended dose of menogaril is 50 mg/m²/day for 5 consecutive days administered as a 2-hour intermittent infusion, repeated every 28 days. [Cancer Treat Rep 70:721–725, 1986]

The clinical utility of doxorubicin and daunorubicin, the two active and commercially available anthracycline antineoplastic agents, is limited because of tumor resistance and cumulative dose-related cardiotoxicity (1,2). Menogaril, a semisynthetic analog of the anthracycline antibiotic nogalomycin (3), was brought to phase I clinical testing in an attempt to find a more active and less toxic anthracycline drug. The mechanism of action for menogaril is not precisely known. Menogaril demonstrates major differences in cellular uptake and localization when compared to doxorubicin and daunorubicin. Menogaril achieves intracellular concentrations >200 times those in the surrounding medium, with only 13% of the drug localized in the nucleus (4). At equivalent extracellular concentrations, the intracellular concentration of daunorubicin would be approximately 10%–20% that of menogaril, with most drug concentrated in the nucleus (5). Menogaril is not cell phase specific and is equally lethal to cells in all phases of the cell cycle (6).

In preclinical in vivo screening, menogaril proved effective against P388 and L1210 leukemias, B_{16} melanocarcinoma, CD_8F mammary carcinoma, and colon 38 carcinoma (7,8). There are also preclinical data that menogaril, when administered orally, is absorbed through the gastrointestinal tract and retains its antitumor activity (9).

In addition to having documented antitumor activity, menogaril did not reduce cardiac guanylate cyclase, an enzyme whose activity is decreased by doxorubicin and daunorubicin and which may be related to the mechanism of anthracycline-induced cardiotoxicity (10,11). In vivo menogaril was less than one-fifteenth as potent as doxorubicin in inducing cardiomyopathy when both drugs were continuously administered to rabbits (12).

The objectives of this phase I trial were to establish the maximum tolerated dose of menogaril when given as a single, intermittent iv infusion daily for 5 days, to evaluate all drug toxic effects, and to document any antitumor activity that might occur.

MATERIALS AND METHODS

Patient Selection and Evaluation

All patients had histologic proof of malignant disease

[1]Received Sept 18, 1985; revised Dec 16, 1985; accepted Dec 24, 1985.

[2]Supported by contract N01-CM-27541 (Division of Cancer Treatment) and Public Health Service grants CA-32107 and CA-07753 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Divisions of Medical Oncology and Developmental Therapeutics, University of Maryland Cancer Center, University of Maryland School of Medicine, Baltimore.

Reprint requests to: Dr. David A. Van Echo, Divisions of Medical Oncology and Developmental Therapeutics, University of Maryland Cancer Center, University of Maryland School of Medicine, 22 S Greene St, Baltimore, MD 21201.

for which conventional chemotherapy had proven ineffective and for which no other therapy with established efficacy was available. All patients had a minimum life expectancy of 12 weeks, had a Karnofsky performance status of at least 60%, and were at least 4 weeks removed from previous chemotherapy (8 weeks for nitrosoureas) and radiotherapy. All patients had adequate marrow function (wbc count $> 3500/\mu l$ and platelet count $> 100,000/\mu l$), adequate liver function (bilirubin < 2.0 mg/100 ml), and adequate renal function (serum creatinine < 2.0 mg/100 ml). Patients were excluded from study if they had congestive heart failure or had previously received > 450 mg/m^2 of doxorubicin. Baseline studies obtained before treatment included hematocrit, wbc count with differential, platelet count, urinalysis, and serum urea nitrogen, creatinine, glucose, alkaline phosphatase, total bilirubin, aspartate aminotransferase, glutamic pyruvate transferase, calcium, and phosphorus. In addition, all patients had an initial chest roentgenograph, ECG, and, during the first day of the first course, a cardiac Holter monitor. Following chemotherapy, patients had weekly assessments which included physical examination, weight, performance status, cbc, and evaluation of drug toxicity and response. All other laboratory work was obtained every 4 weeks. Initially, all patients had serial exercise multigated analysis (MUGA) scans, but the routine use of this test was discontinued unless more than two courses of menogaril were administered or if congestive heart failure was suspected on clinical grounds.

All patients entered on this study were informed of the investigational nature of the treatment, and an informed consent approved by the Institutional Review Board was signed. Measurable disease was not required but was measured serially when present.

Drug Schedule and Administration

Menogaril was supplied by the Investigational Drug Branch of the National Cancer Institute (Bethesda, MD). Each vial, containing 50 mg of menogaril, 100 mg of mannitol, and 16.6 mg of L-lactic acid, was reconstituted with 10 ml of sterile water. The appropriate dose of menogaril was dissolved in 150 ml of 5% dextrose in water and infused over 1 hour. For doses of 42 mg/m^2/day and higher, menogaril was dissolved in 250 ml of 5% dextrose in water, and the infusion duration was prolonged to 2 hours. Each bottle was formulated immediately prior to the start of the infusion.

The starting dose was 3.5 mg/m^2 daily for 5 days, which approximated one-third of the toxic dose low in dogs. Doses were escalated to 56 mg/m^2/day $\times$ 5 with a modified Fibonacci schedule. Treatment was repeated every 28 days provided that the patient had recovered from any drug toxicity and had at least stable disease. Escalation of a dose in an individual patient was permitted after that patient had received two courses at the initial dose with minimal toxicity. Six or more patients were studied at dosages which produced acceptable, reversible toxicity. Patients did not receive any antiemetics during their first course of menogaril, so that nausea and vomiting might be evaluated adequately.

RESULTS

Patient Population

Twenty-seven patients received 54 courses of menogaril, 50 of which were evaluable for toxicity (table 1). For the four inevaluable courses, one patient required emergency radiation therapy, one patient refused further follow-up after administration of his second course of menogaril, one patient died from progressive lymphangitic pulmonary metastases from breast carcinoma 13 days after receiving a second course of menogaril, and one patient refused Days 4 and 5 of therapy of her fourth course of menogaril. One patient, who received 50 mg/m^2/day, died on Day 18 of myocardial infarction which appeared unrelated to drug administration but is included in the evaluation of drug toxicity. There were eight females and 19 males with a median age of 58 years (range, 35-75) and a median

TABLE 1.—Patient characteristics*

Characteristic	
Entered	27
Females/males	8/19
Median age in yrs (range): 58 (35-75)	
Median performance status (range): 80% (60%-100%)	
Previous treatment	
Chemotherapy only	17
Radiotherapy only	2
Chemotherapy + radiotherapy	4
No prior therapy	4
Tumor type	
Biliary tract	1 (0)
Breast	2 (2)
Colon	7 (7)
Gastric	1 (1)
Head and neck	
Squamous cell carcinoma	2 (2)
Lung	
Adenocarcinoma	3 (3)
Squamous cell carcinoma	1 (0)
Malignant melanoma	1 (1)
Ovarian	1 (1)
Pancreatic	1 (0)
Renal	2 (2)
Sarcoma (leiomyosarcoma)	2 (2)
Unknown primary	3 (3)

* Values = No. of patients (No. evaluable for response). Criteria for evaluation for response include measurable disease and 1 course of treatment.

performance status of 80% (range, 60%–100%). Four patients were previously untreated, 17 had received prior chemotherapy, two had received prior radiation therapy, and four had received both prior chemotherapy and prior radiation therapy. Table 1 shows the various tumor types treated and the number of patients evaluable for response. The relatively large number of patients with colon cancer reflects the pattern of previously treated patients referred to our institution at the time of this study. Only one patient each was treated at the 3.5-mg/m^2/day and 17.5-mg/m^2/day dose levels because available information from other ongoing clinical trials indicated the safety of those doses and allowed further dose escalation.

Hematologic Toxicity

Myelosuppression, consisting of both leukopenia and thrombocytopenia, was both dose related and dose limiting, with a steep dose-response relationship (table 2). Significant leukopenia was first noted at doses of 31.5 mg/m^2/day (table 2). Leukopenia and neutropenia became progressively worse at doses > 31.5 mg/m^2/day. Two of the four patients treated at 56 mg/m^2/day developed severe neutropenia and were hospitalized for fever. One of these patients, who had an adenocarcinoma of unknown origin, was previously untreated and had a performance status of 90%. During his first course there was a wbc count nadir of 1000/μl. His second course of menogaril produced a wbc count nadir of 400/μl and the patient was admitted for documented bacteremia. The other patient had metastatic carcinoid and previously had been treated with 5-FU and carboplatin. He developed a wbc count nadir of 1100/μl and a platelet count of 28,000/μl during his first course of menogaril and was admitted to the hospital with fever, although no infection was documented. At the dose of 50 mg/m^2/day, three patients developed wbc counts < 1000/μl, but there were no infectious complications. All patients, except one, recovered their wbc counts > 3500/μl by Day 28. Menogaril did not suppress platelet counts as severely as it did wbc counts. In patients treated with a menogaril dose of 50 mg/m^2/day, the lowest platelet count observed was 66,000/μl. Among the four patients who received more than one course of menogaril at 50 mg/m^2/day, there did not appear to be cumulative myelosuppression.

Nonhematologic Toxic Effects

Local reactions to menogaril at the site of infusion were seen initially at the lowest dose level and became progressively more severe with dose escalation (table 3). These reactions, which consisted of local urticaria and moderate to severe phlebitis with blister formation, lasted up to 6 weeks after menogaril administration. A number of maneuvers were employed to reduce or eliminate these reactions. These maneuvers included: (*a*) increasing the dilution of the drug, ie, increasing the volume in which the drug was administered from 150 to 250 ml of 5% dextrose in water; (*b*) changing the time course of infusion from 1 hour to 2 hours; and (*c*) premedicating with steroids and antihistamines. None of these procedures proved successful. Two small extravasations of menogaril occurred and resulted only in mild erythema which resolved within 24 hours. Infusions through central venous catheters produced no clinically detectable phlebitis. There were no significant hepatic or renal toxic effects noted. Mild nausea and vomiting, stomatitis, and alopecia were occasionally observed at doses ⩾ 31.5 mg/m^2/day.

Patients' cardiac rhythms were assessed continuously for 24 hours by Holter monitor on the first day of the first course of treatment. Rhythm recordings were

TABLE 2.—Hematologic toxic effects of menogaril

Dose (mg/m^2/day × 5)	No. of patients	No. of evaluable courses	Wbc count median nadir × 10^3/μl (range)	Platelet count median nadir × 10^3/μl (range)	Median days to nadir (range)	Median days to recovery from Day 1 of treatment cycle (range)
3.5	1	1	5.9	387	9	—
7	3	5	6.2	326	22	—
			(4.2-6.7)	(238-345)	(11-24)	—
11.5	3	6	5.7	202	22	—
			(3.7-8.1)	(149-364)	(15-31)	—
17.5	1	1	7.4	297	10	—
31.5	3	5	2.9	251	18	25
			(1.6-3.7)	(77-368)	(14-23)	(25-28)
42	8	13	2.4	258	15	27
			(1.0-3.8)	(119-580)	(10-20)	(13-33)
50*	6*	13*	2.3	167	16	26
			(0.5-3.7)	(66-319)	(10-18)	(21-43)
56	4	5	1.1	143	17	24
			(0.4-2.1)	(28-376)	(15-19)	(23-46)

* One additional patient died at Day 18 with a wbc count of 0.7 and a platelet count of 25 × 10^3/μl.

TABLE 3.—Nonhematologic toxic effects of menogaril

Dose (mg/m^2/day × 5)	No. of patients	No. of patients/Eastern Cooperative Oncology Group toxicity grade						
		Nausea and vomiting	Alopecia	Stomatitis	Hepatic	Renal	Inflammation at injection site	Phlebitis
3.5	1	0	0	0	0	0	1/I	0
7.0	3	0	0	0	0	0	3/I	1/I
11.5	3	0	0	0	0	0	2/I	0
17.5	1	0	0	0	0	0	0	0
31.5	3	0	1/I	0	0	0	1/I 1/II	1/II
42	8	1/I	4/I	2/I	0	0	1/I 3/II	1/I 1/II 1/III
50	6	1/I	3/I	1/I	0	0	6/II	6/II
56	4	2/I	3/I	1/I	0	0	4/II	1/II

initiated approximately 2 hours before the start of menogaril infusion and served as baseline. One patient was unable to have a Holter monitor because of extensive skin involvement from breast cancer. Arrhythmias that were noted on the monitors did not change appreciably in character or frequency during or after menogaril infusion. No arrhythmias were specifically associated with drug infusion.

Five patients received more than two courses of menogaril, with the highest cumulative dose being 1000 mg/m^2. None of these patients developed any clinical evidence of congestive heart failure or any significant change in the ejection fraction as measured by MUGA scan.

Responses

There were no major objective responses observed among the 24 patients with measurable disease. A mixed response of liver metastases was detected by computerized tomographic scan in a patient with leiomyosarcoma who had previously been treated with doxorubicin. This response lasted 4 months. Another patient, with an adenocarcinoma of unknown origin and no prior therapy, had a 25% reduction in the size of liver metastases. This response, as measured by computerized tomographic scan, lasted 1 month.

DISCUSSION

This clinical trial of menogaril represents part of the organized attempt to delineate an anthracycline which would be less toxic or more effective than the currently available agents from this class of drugs. The dose-limiting toxic effect of menogaril is myelosuppression, consisting primarily of leukopenia, which exhibits a steep dose-response relationship. Drug-induced neutropenia led to hospitalization in 50% of the patients treated at a dose of 56 mg/m^2/day for 5 days. At a dose of 50 mg/m^2/day for 5 days, corresponding to an 11% lower dose, the median wbc count nadir was 2300/μl, and there were no infectious complications related to neutropenia. There was variation in hematologic toxicity among individual patients. Pharmacokinetic studies performed in conjunction with this clinical trial demonstrated that this variation reflected the difference in disposition of menogaril by individual patients. Specifically, the percent decreases in wbc count and neutrophil count were highly correlated with the area under the curve of plasma menogaril concentration versus time (13). Although the number of patients who received myelosuppressive doses of menogaril was small, individual variability in hematologic toxicity did not appear to be related to prior cytotoxic treatment.

Cardiac monitoring of patients in this study revealed a multiplicity of cardiac arrhythmias including asymptomatic ventricular arrhythmias, atrial arrhythmias, and conduction disturbances. These arrhythmias were seen during pretreatment monitoring. Menogaril administration did not appear to induce new arrhythmias or change the character or frequency of the underlying rhythm disturbance. This is consistent with the results of other anthracycline studies (14,15).

In an attempt to define development of congestive cardiomyopathy, serial MUGA scans were performed to assess ejection fractions in patients who received more than two courses of menogaril. Only one patient received a cumulative dose as high as 1000 mg/m^2. There was no evidence of congestive failure. However, there were a small number of patients in this category, and this clinical trial does not constitute evidence for lack of drug-associated cardiomyopathy. Preclinical animal data suggest menogaril is less cardiotoxic than doxorubicin, but this will need to be confirmed in future clinical trials.

Local toxic effects, consisting mostly of phlebitis and skin reaction at the site of infusion, were a major problem. This was increased further by the 5-day infusion schedule employed in this study. These problems were not observed when central venous lines were used.

There have been reports of decreased acute reactions when anthracyclines are reconstituted with saline, but menogaril is insoluble in saline (16). Two small extravasations of menogaril occurred in this study but did not cause ulceration and did not need subsequent debridement. This is in contradistinction to the vesicant reaction expected from doxorubicin and daunorubicin.

For phase II studies, the recommended dose of menogaril is 50 mg/m^2/day for 5 consecutive days (250-mg/m^2 total dose) administered as a 2-hour intermittent infusion, repeated every 28 days. This dose is > 25% the recommended dose of 200 mg/m^2 for the single-dose schedule (17). However, the higher incidence of local reactions observed with repeated administration may make the single-dose schedule more attractive for further clinical trials.

REFERENCES

1. LENAZ L, and PAGE JA. Cardiotoxicity of Adriamycin and related anthracyclines. Cancer Treat Rev 3:111-120, 1976.
2. MINOW RA, BENJAMIN RS, and GOTTLIEB JA. Adriamycin (NSC-123127) cardiomyopathy—an overview with determination of risk factors. Cancer Chemother Rep Part 3, vol 6(2):195-201, 1975.
3. WILEY PF. Improved antitumor activity by modification of nogalomycin. J Nat Prod 42:569-582, 1979.
4. EGORIN MJ, CLAWSON RE, COHEN JL, ET AL. Cellular pharmacology of 7-(R)-*O*-methylnogarol: a new anticancer agent. J Pharmacol Exp Ther 210:229-236, 1979.
5. EGORIN MJ, CLAWSON RE, COHEN JL, ET AL. Cytofluorescence localization of anthracycline antibiotics. Cancer Res 40:4669-4676, 1980.
6. BHUYAN BK, BLOWERS CL, and SHUGARS KD. Lethality of nogalomycin analogs and adriamycin. Cancer Res 40:3437-3442, 1980.
7. NEIL GL, BLOWERS CL, HOUSER DJ, ET AL. Antitumor activity of some new analogs of nogalomycin. Proc Am Assoc Cancer Res and ASCO 18:2, 1977.
8. NEIL GL, KUNTZEL SL, and MCGOVREN JP. Treatment of mouse tumors with 7-con-*O*-methylnogarol and other analogs of the anthracycline antibiotic, nogalomycin. Cancer Treat Rep 63:1971-1978, 1979.
9. MCGOVREN JP. Activity of the anthracycline agent 7-con-*O*-methylnogarol (7-OMEN), administered orally to mice bearing P388 or L1210 leukemia. Cancer Treat Rep 64:727-729, 1980.
10. LEVEY BA, RUIZ E, ROGERSON B, ET AL. Failure of aclacinomycin and 7-con-*O*-methylnogarol (7-OMEN) to decrease cardiac guanylate cyclase activity. Cancer Treat Rep 64:1127-1128, 1980.
11. LEHOTAY DC, LEVEY BA, ROGERSON BJ, ET AL. Inhibition of cardiac guanylate cyclase of doxorubicin and some of its analogs; possible relationship to cardiotoxicity. Cancer Treat Rep 66:311-316, 1982.
12. MCGOVREN JP, NEIL GL, DENLINGER RH, ET AL. Chronic cardiotoxicity studies in rabbits with 7-con-*O*-methylnogarol, a new anthracycline antitumor agent. Cancer Res 39:4849-4855, 1979.
13. EGORIN MJ, VAN ECHO DA, WHITACRE MY, ET AL. Human pharmacokinetics, excretion and metabolism of the anthracycline antibiotic menogaril (NSC 269148, 7OMEN) and their correlation with clinical toxicities. Cancer Res 46:1513-1520, 1986.
14. MAYERNIK D, STEVENSON T, and NEIDHART J. Acute cardiac arrhythmias with phase I and II agents. Proc ASCO 1:C-53, 1982.
15. HERSH M, LINN N, KUHN J, ET AL. Electrocardiographic monitoring of patients receiving phase I cancer chemotherapy. Proc ASCO 4:41, 1985.
16. Clinical Brochure: Menogarol (NSC-269148). Bethesda, MD, National Cancer Institute, July 1983.
17. DODION P, SESSA C, GERARD B, ET AL. Phase I trial of menogaril with a single dose schedule. Proc ASCO 4:C-95, 1985.

Phase II Study of Ifosfamide in Cervical Cancer[1]

C. A. Meanwell,[2,3,*] J. J. Mould,[2] G. Blackledge,[2] F. G. Lawton,[2] N. S. A. Stuart,[2] J. Kavanagh,[2] T. N. Latief,[2] D. Spooner,[2] and A. D. Chetiyawardana[2]

Thirty patients with symptomatic, progressive squamous cell carcinoma of the uterine cervix no longer amenable to surgery or radiotherapy were entered in a phase II study of ifosfamide (IFX). Patients were treated with IFX (5 g/m^2 iv given over 24 hours) and concomitant mesna (total dose, 9.2 g/m^2 iv given over 36 hours) every 21 days. One complete response (duration, 10+ months) and nine partial responses were observed, with an overall median response duration of 6.5 months. The median survival of responding patients was 11 months. Objective response rates for lesions arising in previously irradiated sites (four of 22) were significantly lower than for lesions arising in nonirradiated sites (15 of 28) (P = 0.018). There were two treatment-related deaths: one due to leukopenia-associated infection in a patient with peritonitis and severe central nervous system toxicity and one due to central nervous system toxicity without complicating factors. One other patient developed severe but reversible encephalopathy. In all remaining patients hemorrhagic cystitis and hematological and gastrointestinal toxic effects were predictable and manageable. Treatment was delayed for 1 week due to toxicity on seven of 101 occasions: four of these delays were due to mild, reversible impairment of renal function and three were due to leukopenia. Complete though reversible alopecia occurred in 22 of 30 patients. The results indicate that IFX is active in cervical cancer and deserves further study in this setting. [Cancer Treat Rep 70:727–730, 1986]

Ifosfamide (IFX) is a structural isomer of cyclophosphamide which undergoes similar metabolic transformations in vivo (1). The compound was first introduced into clinical trials in 1970 (2), but treatment was associated with severe urothelial toxicity. Preclinical and clinical studies identified the cause of hemorrhagic cystitis (3) and demonstrated that concomitant administration of mesna could prevent this toxicity (4,5). Subsequent phase II trials of IFX with mesna demonstrated clinical efficacy against a variety of neoplasms without urothelial toxicity (6). Furthermore, IFX causes only moderate reversible myelosuppression (7) and therefore has potential for use in combination with other more myelosuppressive agents. Since cyclophosphamide has activity in cervical cancer (8), IFX was selected as one agent in a series of phase II studies carried out to identify active agents in the treatment of this disease.

The aims of this study were to determine the response rate, duration of response, and toxicity observed in women with recurrent cervical cancer treated with single-agent IFX and to study the effects of prior irradiation on the response of individual lesions.

PATIENTS AND METHODS

Patients

Thirty patients with biopsy-proven advanced or recurrent squamous cell carcinoma of the uterine cervix no longer amenable to control by radiotherapy or surgery were entered in the study. All patients were symptomatic with measurable progressive disease and had received no other chemotherapy for at least 4 weeks and no radiotherapy for at least 8 weeks prior to entry. In all patients, renal function was normal as demonstrated by serum creatinine concentration < 120 μmols/L, serum urea < 8.0 mmols/L, and creatinine clearance > 50 ml/minute. Bone marrow function was

[1]Received Oct 1, 1985; revised Dec 24, 1985; accepted Dec 27, 1985.

[2]West Midlands Cancer Research Campaign Clinical Trials Unit and University of Birmingham Department of Medicine (*C. A. Meanwell, G. Blackledge, F. G. Lawton, N. S. A. Stuart,* and *J. Kavanagh*), and Department of Radiotherapy and Oncology (*C. A. Meanwell, J. J. Mould, G. Blackledge, T. N. Latief, D. Spooner,* and *A. D. Chetiyawardana*), Queen Elizabeth Medical Centre, Edgbaston, Birmingham, England.

[3]Supported by Boehringer Ingelheim (Hospital Division).

[*]*Reprint requests to:* Dr. C. A. Meanwell, West Midlands Cancer Research Campaign Clinical Trials Unit, Clinical Research Block, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, England.

adequate in all patients as determined by total leukocyte count $> 3.5 \times 10^9$/L, platelet count $> 100 \times 10^9$/L, and hemoglobin > 11.0 g/L. Patients entered had no serious medical or psychological contraindication to IFX and mesna treatment and no other history of malignancy.

Patient characteristics are shown in table 1. Two patients had received no treatment prior to entry in the study. One of these patients had abdominopelvic disease with pulmonary metastases, while the other had pelvic disease extending to both sidewalls. Three patients had previously been treated with chemotherapy. One of these patients had received combination cisplatin (total dose, 50 mg/m^2) and etoposide (total dose, 400 mg/m^2) as initial treatment for metastatic cervical cancer. The other two patients had received two courses of combination bleomycin (total dose, 80 mg), doxorubicin (total dose, 100 mg/m^2), and mitomycin (total dose, 12 mg/m^2) for recurrence of cervical cancer. None of the three patients had shown objective response to these treatments.

Methods

Before treatment, measurable disease was identified and documented using plain radiography, abdominal ultrasonography, rectosonography, and computed tomography. Corroborative clinical measurements were performed by the investigators.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Patients entered	30
Mean age in yrs (range): 49 (25-71)	
Median Karnofsky performance status (range): 80% (60%-100%)	
Squamous cell type	30
Histological differentiation	
Good	3
Moderate	14
Poor	13
Mean time from diagnosis to recurrence (mos)	21
Initial treatment	
Surgery (all had subsequent pelvic radiotherapy)	
Cone biopsy	2
TAH/BSO*	1
TAH/BSO/pelvic lymphadenectomy	4
Pelvic radiotherapy	
Brachytherapy alone	8
Teletherapy alone	6
Combined	13
Chemotherapy	
Cisplatin and etoposide	1
None	2
Previous chemotherapy for recurrence after initial radiotherapy	
Bleomycin, doxorubicin, and mitomycin	2

*TAH/BSO = total abdominal hysterectomy/bilateral salpingo-oophorectomy.

Patients were prehydrated with 1 L of iv dextrose saline (4% dextrose with 30 mmols of sodium chloride) over 2 hours. A urinary catheter was inserted and a diuresis established by giving 200 ml of 20% mannitol over 30 minutes. Mesna (1 g/m^2 of body surface area) was then given by bolus injection followed by IFX (5 g/m^2) and mesna (5 g/m^2) in 3 L of dextrose saline infused over 24 hours. Subsequent mesna (3.2 g/m^2 in 1 L of dextrose saline) was infused over the following 12 hours. During treatment, urinary output was maintained > 100 ml/minute using 20% mannitol and, where necessary, iv furosemide. Treatment was administered in the hospital every 21 days. Patients were given two cycles of treatment before evaluation of response. If no disease progression was seen then a maximum of six treatment cycles was given.

No dose reductions were carried out. Treatment was delayed for 1 week if there were signs of bone marrow suppression (wbc count $< 3.5 \times 10^9$/L) or impairment of renal function (creatinine > 120 μmols/L, urea > 8 mmols/L). If renal function failed to improve with delay and/or hydration, no further treatment with IFX was given.

Response, duration of response, survival, and toxicity were defined according to World Health Organization criteria (9).

RESULTS

Response

Ten of the 30 patients showed objective response to treatment. One of these patients achieved complete response and the remainder achieved partial response. Ten additional patients had stable disease and nine had disease progression. The patient receiving IFX as first-line treatment for abdominopelvic and pulmonary disease died from drug effects before response evaluation could be made. The patient receiving IFX as first-line treatment for cervical cancer confined to the pelvis had stable disease with treatment. None of the three patients who had received prior chemotherapy responded to IFX therapy.

In all responding patients reduction in measurable disease was noted within two courses of treatment and maximal response was noted within three courses. The median duration of response was 6.5 months, with complete response in one patient sustained for 13 months. The median survival for responding patients was 11 months.

Fifty lesions were assessed for response in this group of patients. Four of the 22 lesions (18%) arising in previously irradiated sites showed objective response to treatment. Fifteen of the 28 lesions (54%) arising in nonirradiated sites showed objective response to treatment (table 2).

TABLE 2.—Lesion response by irradiation status*

Response	No. of lesions	
	Irradiated	Nonirradiated
Complete	0	9
Partial	4	6
Stable disease	9	9
Disease progression	9	4
Nonevaluable (early death)	0	2
Totals	22	30

*$P = 0.018$ (Fisher exact test, 2-tail).

The mean times to disease recurrence for responding and nonresponding patients were 20.5 months (range, 1-92) and 21.5 months (range, 1-54), respectively. The median pretreatment Karnofsky performance status was 80% in both responding and nonresponding groups. There was no significant difference between responders and nonresponders in terms of pretreatment biochemical or hematological parameters.

Toxicity

Thirty patients received a total of 101 courses of treatment. Toxicity consisted primarily of nausea and vomiting, alopecia, myelosuppression, microscopic hematuria, and disturbance of consciousness (table 3).

Noncumulative myelosuppression was evidenced by leukopenia, with a nadir at 10-14 days following treatment. Total leukocyte count fell to $< 3.0 \times 10^9$/L in nine of 15 patients assessed during this period, but leukopenia delayed treatment for 1 week in only three patients.

Nausea and vomiting, starting approximately 18 hours after the start of treatment, was observed in all patients. Vomiting requiring iv fluid therapy occurred with 14% of all courses given. Tolerable and usually transient diarrhea occurred with 13% of the treatment courses. Macroscopic hematuria was observed in only two patients. There was no evidence of cumulative deterioration of renal or hepatic function with IFX treatment, although four patients required treatment delays of 1 week due to reduction of creatinine clearance values to < 40 ml/minute. Twenty-two patients experienced complete though reversible alopecia.

Transient lethargy was associated with 32% of treatment courses and observed in 16 patients. Somnolence was associated with 10% of treatment courses and observed in eight patients.

Three patients developed a severe and characteristic encephalopathy within 20 hours of treatment. The clinical and electroencephalographic features of this toxic effect have been described elsewhere (10). One of the patients recovered spontaneously after 3 days. One patient developed necropsy-proven colonic perforation at the site of pre-existing stercoral ulceration. In this patient, symptoms of peritonitis were masked by central nervous system side effects, and fatal gram-negative septicemia in association with leukopenia ensued. The third patient became comatose and died. In this patient, death was attributed to central nervous system toxicity, since late clinical features included progressive loss of brainstem reflexes and necropsy revealed no other acute cause of death.

DISCUSSION

A number of chemotherapy agents have demonstrated activity in the treatment of advanced and recurrent cervical cancer (8). Objective response rates for single agents rarely exceed 25%, the duration of response is usually short, and survival benefit is only seen in responding patients. Nevertheless, patients with advanced disease are usually symptomatic and worthwhile palliation may be achieved with systemic treatment.

This study identifies IFX as an active agent in the treatment of cervical cancer, with an overall objective response rate of 33% in 30 patients. The median duration of response (6.5 months) and median survival for responding patients (11 months) compare favorably with corresponding figures from similar early studies of cisplatin therapy in advanced and recurrent cervical

TABLE 3.—Frequency of most severe toxicity observed in all patients

Toxic effect	WHO grade—									
	0		1		2		3		4	
	P	C%	P	C%	P	C%	P	C%	P	C%
Hemoglobin	16	75	6	13	4	7	4	5	0	0
Leukocyte count	13	65	6	14	6	11	3	8	2	2
Platelet count	27	96	3	4	0	0	0	0	0	0
Nausea and vomiting	0	5	2	18	20	63	8	14	0	0
Diarrhea	19	85	7	9	4	5	0	0	0	0
Hematuria	14	72	14	23	0	3	2	2	0	0
Alopecia	2	4	2	8	4	20	22	68	0	0
Infection	26	95	3	4	0	0	0	0	1	1
Consciousness	5	57	16	32	6	8	2	2	1	1

*P = No. of patients; C% = % frequency of toxicity observed with all courses given.

cancer (11). Confirmatory studies of IFX activity in this setting are required.

Recent reports describe the administration of cisplatin-based combination chemotherapy prior to radical local treatment in the primary treatment of cervical cancer (12,13). These studies have not yet demonstrated improved survival in patients treated with neoadjuvant chemotherapy, but the approach offers potential for improving cure rates in some patient groups and further active cytotoxic agents are required. In this study, response rates in lesions arising from previously nonirradiated sites were significantly higher than those in lesions arising from irradiated sites. The observed differential response rates may be the result of irradiation selection of chemoresistant tumor cells or irradiation disruption of vasculature and consequent reduction of drug delivery to the tumor (14). If either of these hypotheses is correct, IFX may be of value in combination with other more myelosuppressive agents as neoadjuvant therapy.

Predictable and manageable hematological, gastrointestinal, and urothelial toxic effects are observed in patients with advanced cervical cancer treated with IFX and mesna. Our observations indicate that central nervous system toxicity with IFX and mesna is usually mild. A discriminant function analysis of data from this and other phase II trials of IFX and mesna indicates that low serum albumin concentration, raised serum creatinine concentration, and the presence of pelvic disease are associated with increased risk of severe central nervous system toxicity. These parameters may be used to identify individual patients who may be given IFX and mesna safely in regimens for the treatment of cervical cancer (15).

REFERENCES

1. COLVIN M. The comparative pharmacology of cyclophosphamide and ifosfamide. Semin Oncol 9(suppl 1):2-7, 1982.
2. DRINGS P. Erfahrungen mit neuartigen *N*-Lost-phosphamidestern. Dtsh Med Wochenschr 95:491-497, 1970.
3. BROCK N, POHL J, and STEKAR J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention—1. Experimental studies on the urotoxicity of alkylating compounds. Eur J Cancer Clin Oncol 17:596-607, 1981.
4. BROCK N, POHL J, STEKAR J, ET AL. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention—3. Profile of action of sodium 2-mercaptoethane sulfonate (mesna). Eur J Cancer Clin Oncol 18:1377-1387, 1982.
5. SCHEEF W, KLEIN HO, BROCK N, ET AL. Controlled clinical studies with an antidote against the urotoxicity of oxazaphosphorines: preliminary results. Cancer Treat Rep 63:501-505, 1979.
6. HILGARD P, HERDRICH K, and BRADE W. Ifosfamide—current aspects and perspectives. Cancer Treat Rev 10(suppl A):183-192, 1983.
7. HOLOYE PY, ANDERSON T, DUELGE J, ET AL. Use and safety of high-dose ifosfamide. Semin Oncol 9(suppl 1):78-86, 1982.
8. DEVITA VT, WASSERMAN TH, YOUNG RC, ET AL. Perspectives on research in gynecologic oncology. Cancer 38:509-525, 1976.
9. WORLD HEALTH ORGANIZATION. WHO Handbook for Reporting Results of Cancer Treatment. WHO Offset Publication No. 48, Geneva, 1979.
10. MEANWELL CA, BLAKE AE, BLACKLEDGE G, ET AL. Encephalopathy associated with ifosphamide/mesna therapy. Lancet I:406-407, 1985.
11. THIGPEN T, SHINGLETON H, HOMESLEY H, ET AL. *Cis*-platinum in treatment of advanced or recurrent squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Cancer 48:899-903, 1981.
12. VAN DER BERG MEL, SUBANDONO AJ, DEOLIVEIRA CF, ET AL. Vincristine (V), bleomycin (B), mitomycin (M), cisplatin (P) as an additive treatment to radiotherapy (RT) in high risk patients with squamous cell carcinoma of the uterine cervix (SCCUC). Proc ASCO 3:45, 1985.
13. VALLE JC, REZENDE MR, WERNECK C, ET AL. Neoadjuvant and adjuvant chemotherapy with adriamycin, bleomycin and cisplatin (ABC) and modified radical hysterectomy in the cancer of cervix, stage III. Proc ASCO 4:125, 1985.
14. BONOMI PD, and YORDAN EL, JR. Chemotherapy of cervical carcinoma. *In* Chemotherapy of Gynecologic Cancer (Deppe G, ed). Alan R. Liss, New York, pp 103-124.
15. MEANWELL CA, BLAKE AE, KELLY KA, ET AL. Prediction of ifosfamide/mesna encephalopathy. Eur J Cancer Clin Oncol. In press, 1985.

Phase II Clinical Evaluation of Doxifluridine[1,2]

P. Hurteloup,* J. P. Armand, P. Cappelaere, R. Metz, P. Kerbrat, R. Keiling, P. Fumoleau, P. Fargeot, S. Schraub, P. Bastit, M. Caudry, C. Cauchie, S. Nasca, E. Pommatau,† M. Hayat, P. Chollet, J. Renard, M. Van Glabbeke, and G. Mathé[3]

For the European Organization for Research on Treatment of Cancer Clinical Cooperative Screening Group

Disease-oriented phase II trials of doxifluridine were performed in advanced colorectal, breast, renal, endometrial, stomach, and ovarian carcinomas. The dose schedule recommended by the phase I trial (12.5 g/m^2 by continuous iv infusion over 6 hours once a week for 3 weeks followed by a 1-week rest) was chosen first: the initial dose was later decreased to 10 g/m^2 due to the fact that several neurotoxic effects were reported. A total of 207 patients were entered: 137 patients who received at least two courses of treatment were evaluable for response. Therapeutic activity was demonstrated in breast cancer [two complete responses (CR) and 13 partial responses (PR) among 42 patients], colon cancer (seven PRs among 35 patients), and rectal cancer (six PRs among 23 patients). Some therapeutic activity was detected in ovarian cancer (one CR among nine patients), endometrial cancer (one PR among five patients), and stomach cancer (one PR among five patients). No significant activity was noticed in renal cancer (one PR among 18 patients). Nonhematological toxicity was evaluated according to World Health Organization criteria. Nausea and vomiting were recorded in 50% of the patients (Grade 3–4 in 5%), diarrhea was recorded in 20% (Grade 3–4 in 5%), and cutaneous and allergic reactions were recorded in 10% (Grade 3–4 in 2%). Myelotoxicity during the first treatment course was mild; median wbc and platelet count nadirs (× 10^9 cells/L) were 4.1 (range, 0.1–11) and 194 (range, 20–482), respectively. Nevertheless, some cases of acute leukopenia and thrombopenia were reported. Consciousness alterations and neurologic symptoms were the major side effects (72 of 173 evaluable patients), since treatment had to be interrupted in 34 patients and four lethal neurotoxic effects occurred. At the same total dose of doxifluridine, the risk of neurotoxicity significantly increases with age and with the weekly dose and to the contrary it decreases with increasing bilirubin level. Although activity was demonstrated, this treatment cannot be recommended because of major neurotoxicity. Further pharmacological studies seem warranted to define the optimal dosage schedule and to obtain a better therapeutic index. [Cancer Treat Rep 70:731–737, 1986]

Doxifluridine (5′-deoxy-5-fluorouridine; 5′dFUR) is a new synthetic 5′deoxy nucleoside derivative synthesized by the binding of a pentose ring to a molecule of 5-FU (1). In animals, iv or oral administration of 5′dFUR shows significant antineoplastic activity in various experimental tumor models. Tested in vivo against several rat and

[1]Received Jan 16, 1985; revised Dec 17, 1985; accepted Jan 6, 1986.

[2]Presented at the 13th International Congress of Chemotherapy, Vienna, Sept 1983.

[3]The following institutions participated in the study: Centre Hospitalier J. Minjoz, Besancon, France; Fondation Bergonié, Bordeaux, France; Hôpital St. André, Bordeaux, France; Hôpital Universitaire St. Pierre, Brussels, Belgium; Centre Jean Perrin, Clermont Ferrand, France; Centre G. F. Leclerc, Dijon, France; Centre Oscar Lambret, Lille, France; Centre Léon Berard, Lyon, France; Institut Paoli Calmettes, Marseille, France; Centre Paul Lamarque, Montpellier, France; Centre René Gauducheau, Nantes, France; Centre Antoine Lacassagne, Nice, France; Institut Curie, Paris, France; Hotel Dieu, Paris, France; Institut Jean Godinot, Reims, France; Centre Eugène Marquis, Rennes, France; R.R.T.I., Rotterdam, The Netherlands; Centre Henri Becquerel, Rouen, France; Centre René Huguenin, Saint Cloud, France; C.R.O., St. Etienne, France; Hôpital Civil, Strasbourg, France; C.M.C. Foch, Suresnes, France; Centre Claudius Regaud, Toulouse, France; Centre Alexis Vautrin, Vaudoeuvre les Nancy, France; Institut de Cancérologie et d'Immunogénétique, Villejuif, France; Institut Gustave Roussy, Villejuif, France; and EORTC Data Center.

†Deceased.

*_Reprint requests to:_ P. Hurteloup, Clinical Screening Cooperative Group, European Organization for Research on Treatment of Cancer (EORTC), Institut de Cancérologie et d'Immunogénétique (ICIG), Hôpital Paul-Brousse 14, av. Paul-Vaillant-Couturier, 94804 Villejuif Cedex, France.

murine tumor lines, 5′dFUR appeared to be superior to other fluoropyrimidines in current clinical use, in particular 5-FU (2), the reference antimetabolite used as first-line treatment for various tumors since 1957 (3). Cytotoxic activity of 5′dFUR requires its cleavage to 5-FU. This cleavage occurs via a nucleoside phosphorylase (4), the activity of which differs in normal and neoplastic cells. Therapeutic activity appears to be enhanced by selective distribution in bone marrow and various tumor cells (4,5). 5′dFUR probably acts as a 5-FU prodrug; used at standard dosages, the principal toxic effects of 5-FU include gastrointestinal intolerance, mucosal lesions, bone marrow suppression, alopecia, dermatitis, and, in some cases, anginal pain. When used at relatively high dosages, 5-FU is also neurotoxic (6–11). Experimental side effects with 5′dFUR are very similar to those seen with 5-FU. Compared with 5-FU and other pyrimidine derivatives, 5′dFUR is characterized by a higher therapeutic index in animal species. On the basis of these experimental results, Gouveia et al (12) carried out a clinical phase I trial with weekly iv administration (once weekly for 2–4 weeks, followed by a 1-week rest). Granulocytopenia constituted the dose-limiting toxic effect, and transient neurotoxicity was observed when 5′dFUR was administered as a bolus injection. The minimal toxic dose was 10 g/m^2, the maximal tolerated dose was 15 g/m^2, and the recommended dose schedule was 12.5 g/m^2 once weekly for 3 weeks by continuous iv infusion over 6 hours.

Based on experimental and phase I clinical trial data, the Clinical Screening Group of the European Organization for Research on Treatment of Cancer (EORTC) initiated several disease-oriented phase II studies of 5′dFUR in advanced solid tumors in March 1982. This trial was closed to entry in March 1984.

MATERIALS AND METHODS

This study was confined to patients with histologically demonstrated advanced solid tumors of the breast, colonrectum, kidney, ovary, endometrium, or stomach. Patients who were previously treated with 5-FU were eligible. All patients had documented progressive disease. The presence of measurable and/or evaluable lesions was required to monitor response. Patients with ascites, pleural effusion, or bone metastases as the unique lesion were ineligible. Patients with disturbances limited to biochemical changes and/or increased plasma carcinoembryonic antigen levels were excluded. Eligibility criteria included performance status of 0–2 [World Health Organization (WHO)], life expectancy > 3 months, polymorphonuclear neutrophil count > 2000/mm^3 and/or platelet count > 100,000/mm^3, and normal serum bilirubin and creatinine. Informed consent was obtained according to regulations of each participating institution. Exclusion criteria included age > 75 years, radiotherapy and/or chemotherapy < 3 weeks before the beginning of the study, other concurrent malignancies, uncontrolled infection, central nervous system metastases, cerebrovascular disorders, and/or central nervous system lesions. A two-stage sampling plan was adopted for each disease category, with the probability of rejection of a drug active in ≥ 20% of the patients being < 5%. In the absence of complete or partial response in a specific group, the trial could be discontinued after treatment of the first 14 patients. Otherwise, further inclusion was to be continued to a maximum of 25 patients (13).

5′dFUR was supplied by Hoffmann-La Roche Inc, in vials containing 1 or 5 g of the pure substance as 10% sodium salt. Immediately prior to use, contents of the vials were dissolved in 10 ml of sterile water to obtain an injectable preparation. The dosage schedule recommended by the phase I study was used first: 12.5 g/m^2 once a week for 3 weeks followed by a 1-week rest. Treatment was then restarted on Day 29 and followed the same schedule as above. Due to the appearance of severe neurotoxic effects, the initial drug dose was subsequently decreased to 10 g/m^2.

The drug was administered by continuous iv infusion over 6 hours. Provisions were made for dose reduction as a function of the degree of bone marrow suppression during the previous course of treatment. Therapeutic activity was evaluated for measurable disease according to WHO criteria as follows.

Complete response (CR) was defined as the disappearance of all known disease, determined by two observations ≥ 4 weeks apart. Partial response (PR) was defined as a ≥ 50% decrease in total tumor size of the lesions which have been measured to determine the effect of therapy by two observations ≥ 4 weeks apart. In addition, there could be no appearance of new lesions or progression of any lesion. Progressive disease was defined as a ≥ 25% increase in the size of one or more measurable lesions, or the appearance of new lesions (14). No change was defined as disease in which there was neither progression nor PR. Patients who did not receive two complete treatment cycles (six injections) were not evaluable for response, unless there was evidence of clear-cut disease progression (50% increase after at least three injections).

The toxicity evaluation was done according to WHO criteria. With regard to neurotoxicity a three-level scale was selected to obtain a more appropriate grading: 0 = no neurotoxicity; 1 = mild neurotoxicity with treatment confirmation; and 2 = severe neurotoxicity requiring treatment interruption. For consistency in the recording and reporting of results, the trial coordinator visited each trial center and reviewed each patient file with the clinical investigator in charge.

RESULTS

Therapeutic Activity

Eighteen (8.1%) of 207 patients initially admitted to the study were excluded because of noneligibility. Fifty-

two eligible patients who were not fully evaluable were excluded from the final analysis of response: 11 patients with breast cancer; 11 with kidney cancer; ten with colon cancer; ten with rectal cancer; four with ovarian cancer; four with endometrial cancer; and two with stomach cancer. Reasons for not being fully evaluable are as follows: 13 patients received only a single course of treatment before dropping out due to toxicity; 12 were early deaths unrelated to drug toxicity; 25 were excluded because of protocol violations or incomplete data; and two were early deaths related to the drug.

One hundred thirty-seven patients could be evaluated for efficacy. The distribution of tumor types allowed separate analysis for breast, colon, rectal, and renal cancers. The inclusion of patients was prematurely interrupted due to toxicity and did not allow definitive evaluation of other tumor localizations despite objective responses in some of these groups.

Forty-two patients with breast carcinoma were considered in this analysis. Characteristics are shown in table 1, stratified by initial dosage level. No significant differences were observed between patient characteristics in the two therapeutic regimens except for the 5-FU-pretreated patients; these patients were significantly more frequent in the 10-g/m^2 arm (P = 0.01). A CR or PR rate of 36% was reported in 15 of 42 evaluable patients, with a 95% confidence interval of 21%–50%. In nonevaluable patients a 28% response rate was observed (15 responders among 53 patients), with a 95% confidence interval of 16%–40%. Although the response rate was not significantly different between the two initial dose levels, no conclusions can be drawn from this comparison because of the small number of patients and the fact that the treatments were not randomized.

Thirteen of 15 responding patients had soft tissue lesions: primary tumors, four (one CR; three PRs); skin, ten (two CRs; eight PRs); and lymph nodes, four (two CRs; two PRs). Two PRs were seen in hepatic metastases (table 2). Among the 15 responders, 14 had previously received 5-FU. One patient received the drug as adjuvant treatment (18 g/m^2) and 13 as palliative treatment (median dose, 15 g/m^2). The median time to response was 7 weeks (range, 3–11), while the median duration of response was 26 weeks (range, 4–32). 5′dFUR was discontinued because of disease progression (seven patients), toxicity (three), refusal of further treatment (two), weariness (two), and secondary protocol violation (one).

Thirty-five patients with colon carcinoma could be evaluated for antitumor effects. Patient characteristics are described in table 3. Patient characteristics for the 10-g treatment group (21 patients) and for the 12.5-g treatment group (14 patients) were similar. Eighteen of 35 patients had not received any previous chemotherapy. In the 17 patients previously treated by cytotoxic agents, ten had received 5-FU (table 3). Seven of 35 evaluable patients achieved PR (20%, with a 95% confidence interval of 7%–33%) (table 4); two patients had been pretreated with 5-FU (one as adjuvant treatment, 22 g/m^2; one as palliative treatment, 10 g/m^2). Responses were observed in soft tissue lesions (four patients), pulmonary metastases (two), and hepatic metastases (one). One of these patients achieved CR of lung metastases, while the hepatic

TABLE 1.—Patient characteristics: breast cancer*

Characteristic	5′dFUR (g/m^2)	
	10	12.5
No. of evaluable patients	24	18
Age (yrs)		
Median	55	52
Range	37–73	36–73
WHO performance status		
0–1	17	11
2	7	7
Prior therapy		
Radiotherapy	18	14
Chemotherapy	23	17
5-FU	21	9
No. of prior drugs		
Median	5	5
Range	3–9	3–7
< 3 drugs	5	3
> 3 drugs	18	14

* Unless otherwise specified, values = No. of patients.

TABLE 2.—Therapeutic activity: breast cancer*

Indicator lesions	Response			
	CR	PR	Failure	Total
Skin metastases	2	8	13	23
Nodes	2	2	5	9
Primary tumor	1	3	3	7
Liver	—	2	5	7
Lung	—	—	8	8

* Values = No. of patients.

TABLE 3.—Patient characteristics*

Characteristic	Tumor		
	Colon	Rectum	Kidney
No. of evaluable patients	35	23	18
Age (yrs)			
Median	52	54	59
Range	33–72	38–73	37–75
WHO performance status			
0–1	25	15	12
2	10	8	6
Prior therapy			
Radiotherapy	5	13	6
Chemotherapy	17	10	4
5-FU	10	7	0
Initial dose (g/m^2)			
10	21	7	13
12.5	14	16	5

* Unless otherwise specified, values = No. of patients.

TABLE 4.—Therapeutic activity: response to 5'dFUR*

Tumor	Response		
	PR	No change	Disease progression
Colon			
10 g/m²	6	8	7
12.5 g/m²	1	4	9
Total	7†	12	16
Rectum			
10 g/m²	1	5	1
12.5 g/m²	5	9	2
Total	6‡	14	3
Kidney			
10 g/m²	1	6	6
12.5 g/m²	0	1	4
Total	1§	7	10

*Values = No. of patients.
†Mean confidence interval, 20%; range, 7%–33%.
‡Mean confidence interval, 26%; range, 8%–44%.
§Mean confidence interval, 5%; range, 0%–16%.

lesion remained stable. Responses appeared after two cycles (7 weeks), with a median duration of 26 weeks (range, 12–80+). Treatment was stopped in responding patients for the following reasons: toxicity (five patients), poor physical condition (one), and weariness (one).

Twenty-three patients with rectal carcinoma were evaluated for response. Ten patients had received prior chemotherapy, seven of which consisted of 5-FU (table 3). There were six PRs (26%, with a 95% confidence interval of 8%–44%) (table 4). The indicator lesions were pulmonary metastases (three PRs), hepatic metastases (two PRs), and soft tissue lesions (one CR; one PR). Median time to response was 8 weeks (range, 3–14). The median duration of response was 19 weeks (range, 5+ to 34+). Treatment was discontinued due to progression of disease (three patients), refusal of further treatment (one), orthopedic surgery (one), and neurotoxicity induced by a 2-hour infusion (one). None of the responding patients received previous 5-FU.

Eighteen patients with renal carcinoma evaluable for response were analyzed, 14 of whom had not received prior chemotherapy (table 3). There was one PR in metastatic lymph nodes (table 4), appearing on the 8th week and continuing for 18 weeks. This patient was resistant to multiple-drug chemotherapy including doxorubicin, vincristine, and methotrexate. Including the nonevaluable patients in the calculation of the response rate, the percentage of responding patients is shown in table 5.

Some therapeutic activity was observed in other tumor types: ovarian carcinoma—one CR lasting 40 weeks (axillary lymph node) of nine evaluable patients previously treated; stomach carcinoma—one PR lasting 24 weeks (liver metastasis) of five evaluable patients previously treated; and endometrial carcinoma—one PR lasting 15 weeks (pulmonary metastasis) of five evaluable patients previously treated.

Toxicity

One hundred seventy-six of 207 patients entered in the study could be evaluated for nonhematologic toxicity, while 133 patients were considered for hematologic side effects. Although results of the phase I trial suggested that granulocytopenia was the dose-limiting toxic effect, with 12.5 g/m² only mild to moderate toxicity was observed. Median nadir values for the first course of therapy were as follows: polymorphonuclear neutrophil count, 2.5×10^9 cells/L (range, 0–7.2); wbc count, 4.1×10^9 cells/L (range, 0.1–11.5); and platelet count, 195×10^9 cells/L (range, 20–484). These nadirs occurred on Day 15 (median value based on weekly blood cell counts).

Nevertheless, there were ten cases of acute bone marrow suppression (wbc counts < 1000/mm³ and/or platelet counts < 50,000/mm³), two of which were lethal due to bleeding or pulmonary infection. The median nadir values were similar at both dosages. The distribution of the nadir values observed during the first course (based on weekly blood cell counts) is given in table 6.

Nonhematologic side effects (table 7) were nausea and vomiting (50%), neurotoxicity (42%), diarrhea (20%), allergic and cutaneous reactions (10%), asthenia (9%), and mucositis (5%).

Nine cases of alopecia (one, Grade 3) were reported in 175 patients; however, the rate is difficult to assess in view of prior chemotherapy.

Cardiac toxicity was noted in one 74-year-old man with a good performance status. Twenty-four hours after the first injection (12.5 g/m²), he presented acute precordial pain which resolved spontaneously. The second injection induced the same phenomenon, and the ECG showed a prolongation of the QT segment with a flattening of the T wave. These cardiac disorders again resolved spontaneously; 1 week later, 5-FU was administered (1 g) without further problems.

TABLE 5.—Response rate

Tumor	No. of patients		Response rate (%)	95% confidence interval range (%)
	Responding	Total		
Colon	7	45	15	5–26
Rectum	6	43	13	4–24
Kidney	1	29	3	0–10

TABLE 6.—Occurrence of myelosuppression (1st course)*

	Dose (g/m^2)	
	10	12.5
Total No. of patients	81	52
Wbc count	40 (6)	25 (8)
Platelet count	5 (3)	6 (4)

*Weekly hematology data were available for 133 patients. Values in parentheses = No. with WHO Grade 3-4.

Allergic and cutaneous reactions were noted in 17 patients after a median cumulative dose of 60 g/m^2 (range, 25-135). Principal cutaneous manifestations included eight allergic reactions (five diffuse erythema, two pruritus, and one hand-foot syndrome), seven dry desquamations, and two hyperpigmentations. There were three cases of conjunctivitis. One of these patients had already presented a similar allergic reaction to 5-FU.

Seventy-two patients presented neurotoxicity, which was severe enough to motivate discontinuation of treatment in 34 patients: at 12.5 g/m^2, 31 of 73 patients (42%) presented neurotoxicity, requiring discontinuation of treatment in 18 (25%); and at 10 g/m^2, 41 of 100 patients presented neurotoxicity, with withdrawal of treatment in 16 (16%) (table 8).

Seventeen cases of disturbances in memory and orientation were reported. In 34 patients, cerebellovestibular syndrome was a predominant factor in the neurological clinical evaluation.

Four neurotoxic deaths were recorded. These were observed after various cumulative doses (10, 37.5, 37.5, and 60 g/m^2), with intervals between the last treatment and death of 1, 7, 24, and 66 days, respectively. There was spontaneous recovery in two of six cases of coma (table 9).

The incidence of neurotoxicity clearly increased with the total dose of 5'dFUR administered. In our study, this dose varied widely from patient to patient, and treatment was discontinued in most patients for reasons other than neurotoxicity. We have therefore estimated the risk of neurotoxicity as a function of total administered dose using the Kaplan-Meier method (15) and the hazard rate of the risk curve using the Gehan method (16). Figure 1 shows the risk of neurotoxicity with different total dosages as follows: 5% at 36 g/m^2; 25% at 72 g/m^2; and 45% at 108 g/m^2. The hazard rate was not found to increase significantly with the total dose, which means

TABLE 7.—Occurrence of toxic effects*

	Dose (g/m^2)		
	10	12.5	Total
Total No. of patients	100	76	176
Toxic effect			
Nausea and vomiting	48 (4)	40 (5)	88 (9)
Diarrhea	17 (2)	19 (7)	36 (9)
Allergy/skin reaction	10 (1)	7 (2)	17 (3)
Asthenia	13	3	16
Mucositis	3 (1)	6 (2)	9 (3)
Local	5	5	10
Alopecia	4	5 (1)	9†
Cardiac	—	1 (1)	1 (1)

*Values in parentheses = No. with WHO Grade 3-4.
†Of 175 evaluable patients.

that no cumulative effect could be proven. Risk factors for neurotoxicity have been analyzed with the Cox regression method (17) adjusting for the total dose. Young patients and those with impaired liver function showed significantly less neurotoxicity than did others ($P = 0.005$ and $P = 0.034$, respectively; log-rank test). Furthermore, significantly less neurotoxicity was seen with the lower weekly dose (10 g/m^2) ($P = 0.043$; log-rank test) in patients with normal liver function. Qualitative assessment of symptoms of neurotoxicity showed a particularly high incidence of cerebellovestibular involvement, alone or in conjunction with other signs of toxicity (table 7). All patients presenting neurological symptoms were investigated to exclude causes other than 5'dFUR toxicity. In the presence of drug-related toxicity, the EEG generally showed a slow, generalized background activity with abnormal bilateral frontal activity. Three isotopic cysternographic studies were performed and showed cerebrospinal fluid resorption disorders.

DISCUSSION

We have sought to evaluate the therapeutic efficacy of 5'dFUR in solid tumors, in particular advanced breast, colon, and rectal carcinomas. Some antitumor effects were also seen in ovarian, endometrial, and gastric cancers. No activity was detected in renal cancer.

In this trial, 5'dFUR showed the same spectrum of activity as 5-FU. The results concerning rectal cancer confirm those reported by Abele et al (18), whereas in colon cancer we detected some drug activity, which was not reported by Abele.

TABLE 8.—Occurrence of neurotoxicity*

	Treatment discontinuation required		
Dose (g/m^2)	No	Yes	Total
10	25	16 (40)	41/100 (41)
12.5	13	18 (42)	31/73 (42)

*Values = No. of patients (%).

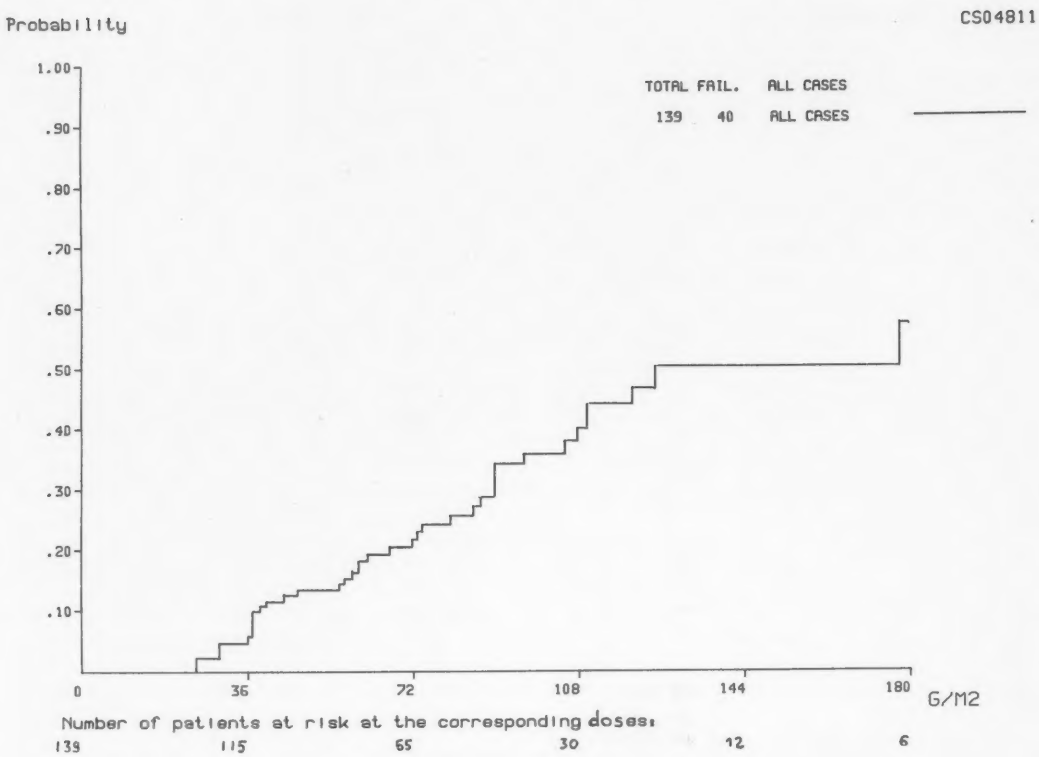


FIGURE 1.—Risk of neurotoxicity.

Along with the assessment of activity, this study demonstrates severe neurotoxicity of 5′dFUR using our protocol of administration. Similar neurotoxicity has been reported with high doses of 5-FU, and the mechanism for this phenomenon has not been completely elucidated (3,4,6). As described for 5-FU, 5′dFUR was taken up into the cerebrospinal fluid. Gouveia et al (12) showed that 1 hour after iv injection of 5′dFUR, 5-FU and 5-FUH_2 were all found in the cerebrospinal fluid. Malet-Martino et al (19) and Bernadou et al (20) indicated that alpha-fluoro-beta-ureido-propionic acid and the fluorure anion were detected by nuclear magnetic resonance in three of six cerebrospinal fluid specimens obtained from patients presenting neurotoxicity. At present, no metabolite has been identified which can be considered as being responsible for neurotoxicity.

Analysis of prognostic factors indicates that neurotoxicity is dose-related. This is supported by a case of overdose in this study. After administration of an initial dose of 18 g/m^2, a 66-year-old man presented with a major cerebellar syndrome of > 1 month's duration, characterized by slow generalized activity on the EEG.

In light of the cellular mechanism for 5′dFUR activation (4), neurotoxicity could be attributed to intracellular liberation and accumulation of 5-FU or other toxic metabolites. The beneficial influence of impaired liver

TABLE 9.—Qualitative evaluation of neurotoxicity: occurrence of the different symptoms*

	Dose (g/m^2)	
	12.5	10
Total No. of patients		
Symptom	73	100
Drowsiness	6 (4)	6 (1)
Sleep disturbance	1	1
Loss of taste	3 (1)	8
Disturbances in memory and orientation	9 (7)	8 (5)
Peripheral neuropathy	—	6
Cerebellar syndrome, isolated	6 (6)	3 (2)
Vestibular syndrome, isolated	5	5 (2)
Vestibular syndrome + central signs	3	5 (2)
Cerebellovestibular syndrome, isolated	1 (1)	3 (2)
Cerebellovestibular syndrome + central signs	3 (3)	—
Coma	3 (2)†	3 (2)†

* Values = No. of patients. Nos. in parentheses = No. with treatment discontinuation.

†Deaths due to neurotoxicity.

function could confirm this hypothesis.

Phase I trials did not report the same major dose-limiting toxicity as was seen in the phase II study. A retrospective analysis of phase I data shows that seven patients were treated at the recommended dose level. These patients received a median of three injections (one treatment cycle), while most neurological disorders reported in phase II appeared only beginning with the second or third treatment cycle (between the 6th and 9th injections). This suggests that in phase I trials with fluoropyrimidines, those patients receiving the recommended dosage should receive several courses of treatment, so as to better evaluate cumulative neurotoxicity.

In view of its manifest activity in this trial, this agent should be subjected to further pharmacologic study to define the optimal dosage schedule and to improve the therapeutic index.

REFERENCES

1. COOK AF, HOLMAN MJ, ET AL. Fluorinated pyrimidine nucleosides. III. Synthesis and antitumor activity of a series of 5′-deoxy-5-fluoropyrimidine nucleosides. J Med Chem 22:1330-1335, 1979.
2. Investigational drug brochure on DFUR. Basel, Switzerland, F. Hoffmann-La Roche and Co, Ltd, 1980.
3. HEIDELBERGER C, CHAUDHURI NK, DANNEBERG P, ET AL. Fluorinated pyrimidines: a new class of tumor-inhibitory compounds. Nature 179:663-666, 1957.
4. ARMSTRONG RD, and DIASIO RB. Selective activation of 5′-deoxy-5-fluorouridine by tumor cells as a basis for an improved therapeutic index. Cancer Res 41:4891-4894, 1981.
5. ARMSTRONG RD, and CADMAN E. 5′-Deoxy-5-fluorouridine selective toxicity for tumor cells compared to human bone marrow. Cancer Res 43:2525-2528, 1983.
6. RIEHL JL, and BROWN WJ. Acute cerebellar syndrome secondary to 5-fluorouracil therapy. Neurology 14:254, 1964.
7. MOERTEL CG, REITEMEIER RJ, BOLTON CF, ET AL. Cerebellar ataxia associated with fluorinated pyrimidine therapy. Cancer Chemother Rep 41:15-18, 1964.
8. KOENIG H, and PATEL A. The acute cerebellar syndrome in 5-fluorouracil chemotherapy: a manifestation of fluoracetate intoxication. Neurology 20:416, 1970.
9. GOTTLIEB JA, and LUCE JK. Cerebellar ataxia with weekly 5 FU administration. Lancet 1:138-139, 1979.
10. BOILEAU G, PIRO AJ, LAHIRI SR, ET AL. Cerebellar ataxia during 5-fluorouracil (NSC-19893) therapy. Cancer Chemother Rep 55:595-598, 1971.
11. BIXENMAN WW, NICHOLLS JVV, and WARWICK OH. Oculomotor disturbances associated with 5-fluorouracil chemotherapy. Am J Ophthalmol 83:789-793, 1977.
12. GOUVEIA J, RIBAUD P, SOMADOSSI JP, ET AL. Phase I study of 5′-deoxy-5-fluorouridine (DFUR). Proc ASCO 26:C103, 1982.
13. GEHAN E. The determination of the number of patients required in a preliminary and follow-up trial of a new chemotherapeutic agent. J Chronic Dis 12:346-353, 1961.
14. WORLD HEALTH ORGANIZATION Handbook for reporting results of cancer treatment. Publication No. 48, Geneva, 1979.
15. KAPLAN E, and MEIER P. Non parametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
16. GEHAN E. Estimating survival functions from the life table. J Chronic Dis 21:629-644, 1969.
17. COX D. Regression methods and life tables. JR Stat Soc B 34:187-220, 1972.
18. ABELE R, ALBERTO P, KAPLAN S, ET AL. Phase II study of doxifluridine in advanced colorectal adenocarcinoma. J Clin Oncol 1:750-754, 1983.
19. MALET-MARTINO MC, MARTINO R, LOPEZ R, ET AL. Detection of fluoropyrimidines and their metabolites in biological samples by fluorine-19 NMR: application to 5′-deoxy-5-fluorouridine. Biomed Pharmacother 37:357-359, 1983.
20. BERNADOU J, ARMAND JP, LOPEZ A, ET AL. Neurotoxicité de la 5′-deoxy-5-fluorouridine: étude par RMN du fluor 19 du liquide céphalo-rachidien chez l'homme. Bull Cancer 71:253, 1984.

Northern California Oncology Group Protocol 6G91: Response to Treatment With Radiation Therapy and Seven-Drug Chemotherapy in Patients With Glioblastoma Multiforme[1]

Victor A. Levin,* William M. Wara, Richard L. Davis, Pamela Silver, Kenneth J. Resser, Kathleen Yatsko, Stephen Nutik, Philip H. Gutin, and Charles B. Wilson[2,3]

The Northern California Oncology Group (NCOG) conducted a nonrandomized phase II study to evaluate the benefit of a seven-drug chemotherapy protocol (NCOG 6G91) in patients with glioblastoma multiforme (GM). Time to tumor progression was the primary end point of the study. The treatment consisted of 5-FU and lomustine administered after surgery and before radiation therapy, hydroxyurea and misonidazole during radiation therapy, and procarbazine and vincristine alternated with carmustine and 5-FU after radiation therapy. Ninety patients entered the study; data from the 64 patients with GM who completed radiation therapy and at least started the postradiation chemotherapy regimen and returned for follow-up examination are analyzed in this report. The median time to tumor progression in the 64 adequately treated patients was 42 weeks; the 25th percentile value was 60 weeks. A Cox multivariate analysis showed that age and extent of surgical resection were important prognostic variables in patients with GM. The results of this treatment regimen were similar to those of a previous NCOG protocol (6G61), which consisted of hydroxyurea during radiation therapy followed by chemotherapy with carmustine or a combination of lomustine, procarbazine, and vincristine. [Cancer Treat Rep 70:739–743, 1986]

The treatment of gliobastoma multiforme (GM) with radiation therapy and chemotherapy after primary surgical resection has proven to be more beneficial than treatment with irradiation alone (7). The most effective agents are still the nitrosoureas and procarbazine (5,7,8,11-13,15,24-26). Although end points such as the time to tumor progression (TTP) and survival have improved slowly but steadily over the years, the prognosis for patients with GM remains dismal. We therefore designed an aggressive chemotherapy program consisting of seven drugs administered before, during, and after radiation therapy.

The treatment was given in three phases, starting with 5-FU and lomustine (CCNU), followed by radiation therapy and concurrent chemotherapy with hydroxyurea and misonidazole, and concluding with procarbazine and vincristine alternating with carmustine (BCNU) and 5-FU. This protocol was based on diverse experimental observations. 5-FU enhances the cytotoxicity of CCNU (18) and may increase the ability of CCNU to kill glioma cells, thereby reducing the tumor cell burden before radiation therapy. Hydroxyurea potentiates radiation-induced cell kill (21,24), improving the response to radiation therapy in patients with GM (16), and misonidazole radiosensitizes hypoxic cells (4,22,23). Procarbazine, vincristine, BCNU, and 5-FU are active against gliomas (7,11,13,14), and tumor cell resistance to one of these drugs does not result in resistance to any of the others.

MATERIALS AND METHODS

Patient Selection

The study was conducted under the auspices of the Northern California Oncology Group (NCOG). Patients

[1]Received June 25, 1985; revised Dec 12, 1985; accepted Jan 6, 1986.

[2]Brain Tumor Research Center of the Department of Neurological Surgery (*V. A. Levin, P. Silver, P. H. Gutin, and C. B. Wilson*), and the Departments of Radiation Oncology (*W. M. Wara*) and Pathology (Neuropathology) (*R. L. Davis*), School of Medicine, University of California, San Francisco; Northern California Oncology Group, Palo Alto (*K. J. Resser* and *K. Yatsko*); and Kaiser-Permanente Foundation Hospitals, Redwood City, CA (*S. Nutik*).

[3]We thank the many members of the Northern California Oncology Group who contributed patients to this study; Margaret Seager, Barbara Turner, and Wanda Ruble for data collection; John Hannigan of the NCOG office for statistical advice and assistance; and Stephen Ordway for editorial assistance.

*Reprint requests to: Victor A. Levin, MD, Brain Tumor Research Center, HSW 783, University of California, San Francisco, CA 94143.

with a histologically verified diagnosis of GM or gliosarcoma, Karnofsky performance scores of 70%-100%, and normal hemograms and blood chemistries were eligible for the study. The diagnosis of GM was based on the histologic appearance of a glial neoplasm with high cellularity, nuclear and cytoplasmic pleomorphism, and vascular endothelial proliferation. Patients erroneously entered before histological review by the reference pathologist (R. L. Davis) and later diagnosed as having anaplastic glioma (AG) continued to receive the therapy but were evaluated separately. Patient enrollment began November 21, 1980, and continued until March 1, 1983. Data obtained as of April 4, 1985, are analyzed in this report.

Treatment Plan

The treatment plan is summarized in table 1. Within 3 weeks after surgery, the patients were given 5-FU (1 g/m^2) by continuous iv infusion on Days 1-3 and CCNU (110 mg/m^2 orally) on Day 7. The second phase of treatment consisted of 4500 rads of whole-brain irradiation with a 1500-rad tumor boost delivered at 180-200 rads/day through lateral opposed portals and concurrent chemotherapy with hydroxyurea (300 mg/m^2 every 4 hours) for four doses on Mondays and Wednesdays; misonidazole (0.5 g/m^2) was given orally 4 hours before each dose of radiation. After completing radiation therapy, the patients entered the final phase of the protocol, during which four drugs were given sequentially for 1 year or until tumor progression. Procarbazine (130 mg/m^2/day) was given orally during Weeks 1-4. This was followed by weekly doses of vincristine (1.4 mg/m^2 iv) during Weeks 5-8. During Week 9, one dose of BCNU (200 mg/m^2 iv) was given; 10-14 days later, 5-FU (1 g/m^2/day) was given by continuous iv infusion for 3 days. Steroid (dexamethasone) doses were kept at the lowest value consistent with good medical practice. Drug doses were reduced for myelotoxicity and for peripheral neuropathy.

TABLE 1.—Treatment schema for NCOG 6G91

Before radiation therapy
5-FU (1 g/m^2/day) on Days 1-3
CCNU (110 mg/m^2 orally) on Day 7
During radiation therapy*
Hydroxyurea (300 mg/m^2 every 4 hrs × 4 doses) on Monday and Wednesday
Misonidazole (0.5 g/m^2) before each radiation therapy fraction
Irradiation, 4500 rads of whole-brain, 180-200 rads/day + 1500-rad tumor boost, 180-200 rads/day
After radiation therapy†,‡
Procarbazine (130 mg/m^2/day orally × 28 days)
Vincristine (1.5 mg/m^2 iv × 1 day/wk × 4) starting 1 wk after completion of procarbazine
BCNU (200 mg/m^2 iv × 1 day) 10-14 days after completion of procarbazine and vincristine cycle
5-FU (1 g/m^2/day × 3) starting 10-14 days after completion of BCNU

*To begin on Monday, 3-7 days after CCNU.

†3 courses of postirradiation chemotherapy were administered. After the 1st course, the sequence was repeated twice, beginning each time with procarbazine 4-5 wks after the last dose of 5-FU.

‡To begin 7-10 days after last radiation therapy fraction.

Evaluation of Response to Therapy

The response to therapy was evaluated from the first dose of 5-FU using criteria developed at the University of California, San Francisco (9,16,19). Before each course of therapy, neurological examination, computerized tomographic brain scan with contrast enhancement, and radionuclide brain scan were performed. Determination of response was based on sequential evaluation of these tests performed after radiation therapy and before each course of chemotherapy. The following definitions were used. Response (score, +2) was defined as improvement in two of the three tests if the dexamethasone dose was stable or decreasing; stable disease was defined as no definite change in two of the three tests; and disease progression (score, −2) was defined as definite worsening in two of the three tests if the dexamethasone dose was stable or increasing.

Quality Control

The histopathologic diagnosis in each patient was reviewed by one of the investigators (R. L. Davis) using published criteria (1,3). To ensure quality and evaluability, radiation portal films and radiation dose schedules were reviewed by the radiation physics section of the NCOG and by the referee radiation therapist (W. M. Wara). Chemotherapy dosing and evaluation criteria were also reviewed (V. A. Levin).

Statistical Analyses and Data Collection

Statistical analyses were performed in the NCOG statistics office. TTP from the start of therapy with 5-FU was the principal end point of the study, which was designed to detect, with 90% power, a 1.5-fold increase (35-52 weeks) in the median TTP when tested at the 1% level. The results were compared with available results from a previous NCOG protocol (6G61), in which patients with GM were treated with hydroxyurea during irradiation, followed by either BCNU or the combination of CCNU, procarbazine, and vincristine (PCV) (14). We were able to compare patients in the present study with historical control patients from NCOG 6G61 because the eligibility criteria, method of evaluation, and personnel were identical in both and the current study was started immediately after the conclusion of the previous study. Because the TTP curves of the patients with GM were exponential in form, the

sample sizes required to detect the stated increase in TTP at the 1% and 5% levels were 80 and 60 patients, respectively (6).

TTP curves were constructed by the method of Kaplan and Meier (10) and compared using the log-rank test (20). Corrected chi-square tests were used to compare the response rates. The method of analysis was patterned after the Cox-Breslow actuarial analysis for censored survival data with covariates (2). In comparing patients with GM in the present study with patients with GM from the previous protocol, the following covariates were considered: Karnofsky score at entry in the study, extent of resection (determined from the operative report), and age.

RESULTS

Ninety patients were entered in the study. Five patients died before completing radiation therapy, five refused chemotherapy after radiation therapy, and five were lost to follow-up review. Eleven patients were found by the referee pathologist to have AG. Thus, there were 64 adequately treated patients with GM in whom TTP and response to therapy could be evaluated. The clinical characteristics of these 64 patients are summarized in table 2. All patients had Karnofsky scores of 70%-100% at entry in the study and were considered good-risk patients. Ten of the patients with AG were adequately treated; TTP and response to treatment in this group were analyzed separately.

The most severe toxic effects among adequately treated patients with GM are listed in table 3. Myelotoxicity was the major dose-limiting factor. Misonidazole-induced peripheral neuropathy was a serious problem, especially in the older patients, and required delay, dose reduction, or discontinuation of vincristine therapy in many patients. Nausea and vomiting were never dose-limiting, but frequently had to be controlled with antiemetics.

The response to therapy among adequately treated patients is summarized in table 4. Data from the most recent analysis of NCOG protocol 6G61 are included for comparison (14). The criteria for evaluability and the definition of the adequately treated group in that study were similar to those in the present study. Completion of radiation therapy and initiation of postirradiation chemotherapy were required in both studies. There was no difference between these two studies in the number of patients who responded and stabilized or in the TTP curves. There were too few patients with AG to make a valid comparison. Four of the patients with AG were responders and five had stable disease. The median TTP for the ten patients with AG was 45 weeks.

Figure 1 shows Kaplan-Meier representations of TTP for patients in the present study and for patients in the previous study (NCOG 6G61) (14) who were treated with BCNU or with PCV. The 6G91 curve was not statistically different from the 6G61 curves by the log-rank test.

Cox proportional hazards analyses were performed on the 6G91 patient set and the 6G91 set versus the comparable 6G61 set to assess the independent prognostic significance of age, sex, Karnofsky score, and extent of resection (biopsy vs subtotal or total resection). Extent of surgery and age were significant covariates among patients with GM in the present study. Biopsy patients did worse than patients who had subtotal or total resection, and younger patients did better than older patients (both, $P = 0.01$). The results of the analysis were similar to those obtained in the previous study

TABLE 2.—Clinical characteristics of 64 adequately treated patients with GM

Characteristic	% of patients
Median age in yrs (range): 56 (29-75)	
Sex	
Male	75
Female	25
Karnofsky score	
70%-80%	40
90%-100%	59
Surgical procedure for primary diagnosis	
Biopsy only	5
Subtotal resection	81
Total resection	14

TABLE 3.—Most severe toxic effects among 64 adequately treated patients with GM

NCOG toxicity criteria	Grade	% of patients
Leukocyte count ($\times 10^3/mm^3$)		
3.0-3.9	1	15
2.0-2.9	2	33
1.5-1.9	3	11
< 1.5	4	8
Platelet count ($\times 10^3/mm^3$)		
100-124.9	1	17
75-99.9	2	15
50-74.9	3	8
< 50	4	8
Gastrointestinal		
Nausea	1	48
Nausea/vomiting that required treatment with antiemetics	2	17
Intractable vomiting	3	6
Severe vomiting that required hospitalization	4	0
Neuropathy		
Decreased deep tendon reflexes, mild pain, or paresthesia	1	6
Absence of deep tendon reflexes, moderate pain, or paresthesia	2	21
Severe weakness, pain, paresthesia, or obstipation	3	11
Paralysis or obstipation requiring surgery	4	0

TABLE 4.—Response to therapy among adequately treated patients with GM in NCOG protocols 6G91 and 6G61

Response	Protocol 6G91 (n = 64)	Protocol 6G61 BCNU (n = 40)	Protocol 6G61 PCV (n = 36)
Complete + partial	15 (23%)	8 (20%)	13 (36%)
Stable disease	47 (73%)	29 (72%)	21 (58%)
Disease progression	2 (3%)	3 (8%)	2 (6%)
Median TTP	41 wks	32 wks*	37 wks*
P value		> 0.4	> 0.07
		> 0.4	

*Refers only to patients with Karnofsky performance score ≥ 70%.

(6G61) (14), except that the extent of surgery seemed to be a stronger covariate in the present study.

DISCUSSION

The benefit of chemotherapy administered after surgery and radiation therapy to patients with supratentorial malignant gliomas is well-established, although the long-term results have been disappointing. In the current study, we used an aggressive regimen of chemotherapeutic agents before, during, and after radiation therapy. The protocol was designed to take advantage of the radiopotentiating effects of hydroxyurea (17) and the hypoxic-cell sensitizing effects of the nitroimidazole (22,23) during radiation therapy for malignant gliomas. To minimize the emergence of tumor cells resistant to the cytotoxic effects of a single drug, agents without known cross-resistance (BCNU, 5-FU, procarbazine, and vincristine) were administered sequentially.

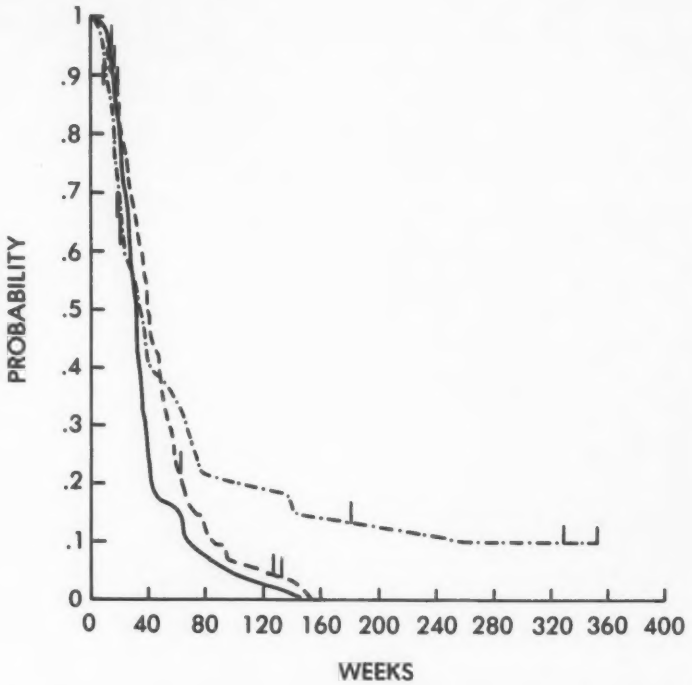


FIGURE 1.—Kaplan-Meier representation of probability of being free of tumor progression for adequately treated GM patients with Karnofsky scores of 70%–100% treated according to protocol 6G91 (n = 64) (---) and those treated with BCNU (n = 30) (———) or PCV (n = 31) (•—•—) according to protocol 6G61. Ticks represent censored patients.

The results of this protocol, the first nonrandomized phase II brain tumor study conducted by NCOG, are disappointing. While the median TTP (41 weeks) was longer than that of the PCV (37 weeks) and BCNU (32 weeks) arms of the previous protocol, NCOG 6G61, which involved fewer drugs (13), the difference between the curves was not statistically significant (fig 1). It is truly unfortunate that our therapeutic program was not more effective. Of positive value, however, is the fact that we were able to conduct a single-arm, nonrandomized study of previously untreated patients with GM by using a control group of patients from the previous NCOG study. This is important because it reduces reliance on randomized studies of new treatments for a cancer that carries a grim prognosis.

The use of historical controls is always controversial. Although we share this concern, several factors were considered to justify this approach in the present study. First, patients with malignant glioma generally do not survive very long. Second, the regional referral pattern of patients with these tumors to our clinical trials has been strong and consistent. Comparing the two studies, we found that the glioblastoma patients were, in fact, similar in terms of important covariates: age, performance status, and extent of surgery. In addition, the TTP curves were exponential. Thus, a reasonable comparison could be made between the present study (6G91) and the two arms of the previous study (6G61). No difference was noted, and the present study was concluded in short order with many fewer patients than would have been required had it been randomized.

The randomized clinical trial is still a necessary part of the clinical evaluation of cancer therapy, but only when the proposed therapy has the potential to significantly increase TTP or the duration of survival. For instance, had our study shown a 50% increase in TTP, we would have considered it necessary to conduct a randomized trial with the NCOG 6G61 PCV arm as control. Because of the time required to design, initiate, and carry out randomized clinical trials in patients with malignant gliomas, it is important that this type of nonrandomized phase II trial be conducted more frequently. Last, given the plethora of new chemotherapeutic agents that are active against central nervous system tumors, it is vital that new combinations of known antineoplastic agents be developed and tested in an expedient manner.

REFERENCES

1. BURGER P, and VOGEL F. Surgical Pathology of the Nervous System and Its Coverings. New York, Wiley Medical Publications, 1982.
2. COX DR. Regression models and life tables. J R Stat Soc 34:187-220, 1972.
3. DAVIS RL, and ERLICH SS. Neuroepithelial tumors of the central nervous system. *In* The Clinical Neurosciences. Neuropathology (Rosenberg RN, and Schochet SS, Jr, eds). New York, Churchill Livingston, 1983, pp 85-113.
4. DISCHE S, SAUNDERS MI, LEE ME, ET AL. Clinical testing of the radiosensitizer Ro-07-0582. Experience with multiple doses. Br J Cancer 35:567-579, 1977.
5. EDWARDS MS, LEVIN VA, and WILSON CB. Brain tumor chemotherapy: an evaluation of agents in current use for phase II and III trials. Cancer Treat Rep 64:1179-1205, 1980.
6. GEORGE SL, and DESU MM. Planning the size and duration of a clinical trial studying the time to some critical event. J Chronic Dis 27:15-24, 1974.
7. GUTIN PH, and LEVIN VA. Surgery, radiation, and chemotherapy in the treatment of malignant brain tumors. *In* Controversies in Neurology (Thompson RA, and Green JA, eds). New York, Raven Press, 1985, pp 67-83.
8. GUTIN PH, WILSON CB, KUMAR AR, ET AL. Phase II study of procarbazine, CCNU, and vincristine combination chemotherapy in the treatment of malignant brain tumors. Cancer 35:1398-1404, 1975.
9. HILL TC, HOFFER PB, and LEVIN VA. A comparison of one- and two-hour delayed brain scans in patients undergoing chemotherapy for primary brain tumors. J Nucl Med 18:877-880, 1977.
10. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
11. KUMAR ARV, RENAUDIN J, WILSON CB, ET AL. Procarbazine hydrochloride in the treatment of brain tumors. A phase II study. J Neurosurg 40:365-371, 1974.
12. LEVIN VA. Chemotherapy of recurrent brain tumors. *In* Nitrosoureas: Current Status and New Development (Prestayko AW, and Crooke ST, eds). New York, Academic Press, 1981, pp 159-167.
13. LEVIN VA, EDWARDS MS, WRIGHT DC, ET AL. Modified procarbazine, CCNU, and vincristine (PCV-3) combination chemotherapy in the treatment of malignant brain tumors. Cancer Treat Rep 64:237-241, 1980.
14. LEVIN VA, WARA WM, DAVIS RL, ET AL. Phase III comparison of BCNU and the combination of procarbazine, CCNU, and vincristine administered after radiation therapy with hydroxyurea to patients with malignant gliomas. J Neurosurg 63:218-223, 1985.
15. LEVIN VA, and WILSON CB. Chemotherapy: agents in current use. Semin Oncol 2:63-68, 1975.
16. LEVIN VA, WILSON CB, CRAFTS D, ET AL. Criteria for evaluating malignant brain tumor patients undergoing chemotherapy. J Neurosurg 47:329-335, 1977.
17. LEVIN VA, WILSON CB, DAVIS R, ET AL. Phase III comparison study of BCNU, hydroxyurea and irradiation for the treatment of primary malignant gliomas. J Neurosurg 51:526-532, 1979.
18. MULDER JH, SMINK T, and VAN PUTTEN LM. Schedule dependent effectiveness of CCNU and 5-fluorouracil in experimental chemotherapy. Eur J Cancer 13:1123-1131, 1977.
19. NORMAN D, ENZMANN D, LEVIN VA, ET AL. Computerized tomographic scanning in the evaluation of malignant gliomas under therapy. Radiology 121:85-88, 1976.
20. PETO R, and PETO J. Asymptotically efficient rank invariant test procedures. J R Stat Soc 135:185-198, 1972.
21. SINCLAIR WK. The combined effect of hydroxyurea and x-rays on Chinese hamster cells in vitro. Cancer Res 28:198-206, 1982.
22. URTASUN R, FELDSTEIN ML, PARTINGTON J, ET AL. Radiation and nitroimidazoles in supratentorial high-grade gliomas: a second clinical trial. Br J Cancer 46:101-108, 1982.
23. URTASUN RC, BAND PR, CHAPMAN JD, ET AL. Mitronidazole as a radiosensitizer. N Engl J Med 295:901-902, 1977.
24. WEISS BG, and TOLMACH LJ. X-ray induced sensitization of HeLa cell to hydroxyurea. Radiation Res 31:579, 1967.
25. WILSON CB, BOLDREY EB, and ENOT KJ. 1,3-Bis(2-chloroethyl)-1-nitrosourea (NSC-409962) in the treatment of brain tumors. Cancer Chemother Rep 54:273-281, 1970.
26. WILSON CB, GUTIN H, BOLDREY EB, ET AL. Single-agent chemotherapy of brain tumors: a five-year review. Arch Neurol 33:739-744, 1976.

Clinical and Pharmacokinetic Studies of Prolonged Administration of High-Dose Uridine Intended for Rescue From 5-FU Toxicity[1,2,3]

C. J. van Groeningen,* A. Leyva, I. Kraal, G. J. Peters, and H. M. Pinedo[4,5]

A clinical and pharmacokinetic investigation of prolonged administration of high-dose uridine was performed in seven patients with advanced-stage cancer. Uridine administration was examined as a continuous infusion at 1 and 2.5 g/m²/hr (two patients) and as a series of intermittent infusions during 72 hrs at doses of 1–3 g/m²/hr, whereby 3-hr uridine administration was alternated with a 3-hr treatment-free interval (six patients). Continuous infusions of uridine resulted in plasma uridine concentrations of 0.5–1 mM, but was discontinued due to rapid increase in body temperature. Further studies focused on the intermittent schedule in an attempt to avoid the development of fever. Intermittent uridine infusion resulted in markedly elevated plasma uridine levels in the millimolar range. However, during the treatment-free period, rapid elimination of uridine was observed, resulting in plasma levels of 138–335 μM for 3 g/m²/hr. Plasma uracil concentrations also increased markedly, but smaller fluctuations compared to uridine were seen. Total urinary excretion of uridine was 15%–40% of the dose, while uracil excretion in urine was 2%–17%. Intermittent uridine infusion resulted in little or no rise in body temperature (≤ 1.0°C) in ten of 12 courses, and fever of > 39°C in two courses. Both intermittent and continuous infusion of uridine gave rise to phlebitis, which necessitated central venous administration. These data show that using an intermittent infusion schedule, long-term administration of uridine is tolerable, with fever being dose-limiting. Intermittent infusion provides for the maintenance of markedly elevated plasma uridine levels and long-term uridine exposure to the tissues, and may be useful in further studies aimed at testing the potential of uridine to rescue patients from 5-FU toxicity. [Cancer Treat Rep 70:745–750, 1986]

5-FU metabolism is complex and may interfere with cellular processes through two main mechanisms. The first involves its conversion to floxuridine 5′-monophosphate, which inhibits thymidylate synthetase, leading to suppression of DNA synthesis (1). Second, 5-FU may be incorporated into RNA as 5-fluorouridine 5′-triphosphate in place of UTP, leading to disruption of normal RNA function and processing (2). It has been demonstrated that the incorporation of floxuridine 5′-triphosphate into DNA may be yet another mechanism of action for 5-FU (3). Modulation of normal pyrimidine metabolism could conceivably alter activity of 5-FU.

It has been demonstrated that the administration of uridine after 5-FU can rescue tumor-bearing mice from 5-FU toxicity, allowing the use of otherwise lethal doses of 5-FU, which results in an enhanced therapeutic index (4,5). Martin et al (4) employed two different schedules in $B6D2F_1$ mice whereby uridine administration was started 2 hrs after 5-FU. Seven doses (800 mg/kg) of uridine on 2 successive days or two doses (3500 mg/kg) of uridine separated by 18 hrs were both effective. Klubes et al (6) administered uridine to $B6D2F_1$ mice as continuous sc infusions during 5 days, starting 24 hrs after 5-FU at a dose of 800 mg/kg. In the latter schedule, different doses of uridine were examined and 1 g/kg was found to be the minimal dose required to rescue mice from 5-FU lethality. The results of these preclinical studies suggest that prolonged exposure to uridine is necessary to achieve rescue from the toxic effects of 5-FU. With the use of

[1]Received May 15, 1985; revised Dec 13, 1985; accepted Jan 7, 1986.

[2]Supported in part by the Netherlands Cancer Foundation (K.W.F. project I.K.A. 83-16).

[3]Presented in part at the 75th Annual Meeting of the American Association for Cancer Research, Toronto, Ontario, Canada, May 9-12, 1984.

[4]Department of Oncology, Free University, Amsterdam, The Netherlands.

[5]We thank Ms. H. E. Gall for her assistance with blood drawing for the pharmacokinetic studies and Mrs. R. Schoemaker for secretarial assistance.

*Reprint requests to: C. J. van Groeningen, MD, Department of Oncology, Free University Hospital, No. V1066A, de Boelelaan 1117, 1077 MB Amsterdam, The Netherlands.

uridine rescue, the therapeutic effectiveness of 5-FU has been enhanced in mice bearing colon tumor 26 (4) or B16 melanoma but not in mice with L1210 leukemia (5).

In different cell types, either the RNA- or DNA-directed actions of 5-FU may be the principal determinants of its cytotoxicity (7). Qualitative differences between tumor and normal cells may explain the ability of uridine to selectively reverse the toxic effects of 5-FU. However, the exact biochemical mechanism of the rescue has not been clearly defined. From studies in the $CD8F_1$ murine mammary carcinoma system, it has been concluded that the protection from 5-FU toxicity afforded by the addition of uridine is due to the reduction of 5-FU in RNA rather than by reversal of the FdUMP block on thymidylate synthetase (8).

Recently, we reported the results of a clinical study on the administration of uridine to humans (9). In the latter study, uridine administered as 1-hr iv infusions at doses of 1-12 g/m^2 was well tolerated; the only toxic effect observed was transient shivering at the highest dose levels. Pharmacokinetic data revealed rapid elimination with a terminal half-life of 118 mins, possibly indicating short-term exposure of the tissues to uridine. With this schedule, no rescue from 5-FU toxicity could be demonstrated in two patients.

The present paper describes the pharmacokinetic and clinical effects of uridine administered as prolonged iv infusion. These data are useful in further clinical studies on the role of uridine in extending the therapeutic index of 5-FU.

MATERIALS AND METHODS

Patients

Data on seven patients with advanced cancer are listed in table 1. Patients were treated with weekly iv bolus injections of 5-FU at doses of 500-864 mg/m^2. All patients were in good general condition (Karnofsky performance status ≥ 70) and had normal renal and bone marrow function. Informed consent was obtained in each case. Six patients were given uridine alone 2 days after 5-FU, while two patients received uridine 3 hrs after 5-FU. Patient 5 received uridine both ways. All patients were hospitalized during treatment.

Uridine Administration

Uridine, obtained from Sigma Chemical Co, St. Louis, MO, was prepared for clinical use and tested for the presence of pyrogens by the Pharmacy Department of our hospital as described earlier (9).

Uridine was given as a continuous iv infusion to the first two patients. In subsequent patients, uridine was given as intermittent iv infusions during a 72-hr period in an attempt to avoid side effects, whereby 3-hr uridine infusions were separated by 3-hr infusion-free intervals. Two of the latter patients also received intermittent iv infusions of uridine in combination with 5-FU as an initial attempt to rescue them from 5-FU toxicity. Constant infusion rates during uridine administration were maintained with the use of an IMED infusion pump. Continuous infusion of uridine was given at doses of 1.0 and 2.5 $g/m^2/hr$, whereas intermittent uridine infusion was studied at doses of 1.0-3.0 $g/m^2/hr$.

Toxicity

During the hospitalization period, patients were monitored for side effects by clinical observation and measurement of body temperature at 3-hr intervals. Thereafter, patients were examined weekly according to medical history, physical examination, cbcs, serum creatinine, and serum liver function tests (bilirubin, alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and lactic acid dehydrogenase).

Uridine Pharmacokinetics

Plasma and urine of patients given uridine were analyzed for uridine and uracil by high-pressure liquid chromatography. For the continuous iv infusions, blood was drawn before the infusion and at 1-hr intervals

TABLE 1.—Characteristics of patients given uridine

Patient No.	Age (yrs)	Sex	Diagnosis	Uridine dose ($g/m^2/hr$)
1	64	F	Colon cancer	2.5,* 2.0, 3.0
2	65	F	Adenoid cystic cancer	1.0*
3	67	M	Adenocarcinoma, unknown primary	1.0
4	70	M	Colon cancer	3.0
5	51	F	Breast cancer	3.0, 3.0,† 3.0,† 3.0†
6	61	F	Breast cancer	3.0
7	57	F	Colon cancer	3.0,† 2.0,† 2.0†

* In these courses, uridine was given as a continuous iv infusion; all other courses were given with intermittent administration.

†Uridine given in combination with 5-FU.

during the infusion. For the intermittent iv infusions, blood was drawn before the infusion and at various time points corresponding with either the start or the end of a 3-hr uridine administration period. Urine was collected at 6-hr intervals during treatment. Plasma was separated from heparinized blood by centrifugation and stored at −20°C. After collection, urine was also stored at −20°C. Uridine and uracil in plasma and urine were determined as described before (9).

RESULTS

Pharmacokinetics of Uridine

Uridine was administered as a continuous infusion in two patients and as an intermittent infusion in six patients. Continuous infusions were at doses of 1 and 2.5 g/m²/hr and intermittent infusions were at doses of 1-3 g/m²/hr (table 1). A total of 14 courses of uridine were given. Plasma concentrations of uridine and uracil were determined in nine courses.

In pretreatment plasma, uridine concentration was 3-6 μM, while uracil concentration was < 1 μM. For the continuous infusions, the concentration versus time curves are depicted in figure 1.

During continuous infusion of 1.0 and 2.5 g/m²/hr, plasma uridine concentrations increased to steady-state levels of 500 and 1000 μM, respectively, which were reached at 6 hours. Plasma uracil concentrations increased to 50 and 280 μM, respectively, by the end of the infusion. Due to side effects, uridine administration was discontinued at 7 and 10 hours. No blood samples were taken after discontinuation of the infusion.

For the intermittent infusion, a representative concentration versus time curve for a dose of 3 g/m²/hr is given in figure 2. Uridine infusion using the intermittent schedule was studied mainly at the 3-g/m²/hr dose (table 2). Peak uridine concentrations (at the end of the 3-hr infusion) amounted to 846-1306 μM, while nadir concentrations (at the end of a 3-hr infusion-free period) were 138-335 μM. Variations in uracil concentrations were much smaller in the individual patients, but marked interpatient differences were observed. Urinary excretion of uridine amounted to 15%-40% of the administered dose, while uracil in the urine was 2%-17% of the dose on a molar basis.

Toxicity of Prolonged Uridine Infusion

In the two courses of uridine administered as a continuous infusion at doses of 1 and 2.5 g/m²/hr, treatment was discontinued at 10 and 7 hrs, respectively, due to fever. Patient 1 developed a body temperature of 39.3°C accompanied by a 1-hr period of shivering. Over a period of 12 hrs, the temperature gradually decreased to normal. Patient 2 developed fever to 38.6°C without shivering. In both patients, the rise in body temperature could be attributed to no cause other than uridine administration. Besides fever, the two patients developed a painful phlebitis at the infusion site. After this first experience with prolonged administration of uridine, it was felt that limiting the length of time of continuous iv infusion might decrease the risk of fever. Therefore, uridine infusion was further studied using

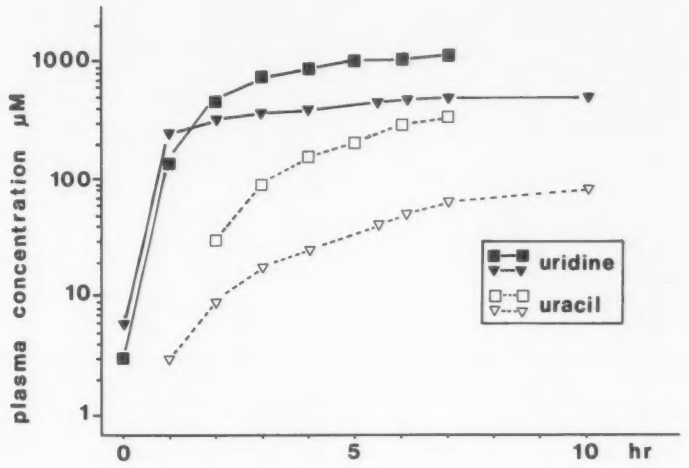


FIGURE 1.—Plasma concentration vs time curves of uridine and uracil in 2 patients receiving uridine as a continuous infusion at doses of 1 (□, ▽) and 2.5 (■, ▼) g/m²/hr.

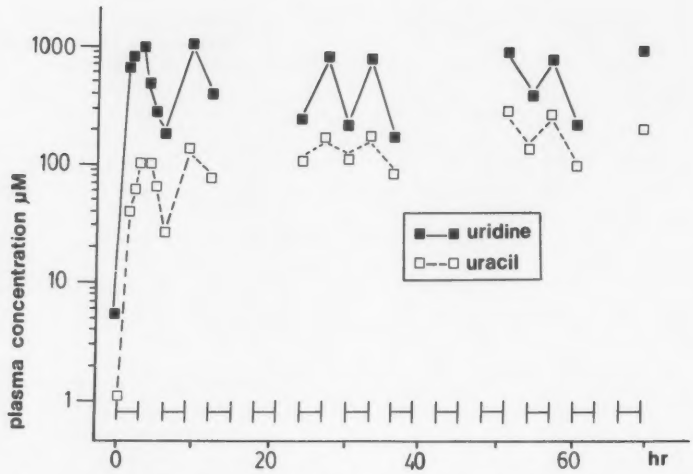


FIGURE 2.—Representative plasma concentration vs time curves of uridine and uracil in a patient receiving uridine as an intermittent infusion at a dose of 3 g/m²/hr during 72 hrs. |——| = a 3-hr infusion period of uridine.

an intermittent schedule (table 3). In the total of 12 courses given as intermittent infusions, four proceeded without any rise in body temperature, while in six courses only a mild increase in body temperature was observed (≤ 1°C). Two courses at doses of 2 and 3 g/m²/hr resulted in a body temperature ≥ 39°C. In Patient 6, who received 3 g/m²/hr, uridine was discontinued 45 hours after the start of infusion.

Phlebitis was also observed in the first patient to receive uridine in the intermittent schedule. Frequent change of infusion site on both arms was required. Subsequently, uridine was given to Patients 4–7 by central venous infusion. No other side effects of either continuous or intermittent uridine administration were encountered.

DISCUSSION

The rationale for the use of high-dose uridine after treatment with 5-FU is that preclinical studies have demonstrated the potential of uridine to selectively rescue normal tissues from 5-FU toxicity. The safe use of higher doses of 5-FU could extend the therapeutic index of this commonly used anticancer agent. Both Martin et al (4) and Klubes et al (5) derived from their studies in mice that the critical factor to achieving rescue was exposure of the tissues to uridine for extended periods rather than high peak plasma concentrations of uridine. A possible explanation proposed is that UTP pools can be expanded only to a maximum of two- to three-fold, regardless of the uridine dose, and that this

TABLE 2.—Plasma concentrations and urinary excretion of uridine (URD) and uracil (URA) of intermittent uridine infusions

Patient No.	Dose (g/m²/hr)	URD concentration (μM)*		URA concentration (μM)*		Urinary excretion†	
		Peak	Nadir	Peak	Nadir	URD	URA
3	1	205 ± 26 (4)	40 ± 6 (3)	13 ± 2 (4)	< 1.0 (3)	15	2
1	2	969 ± 72 (6)	393 (1)	466 ± 50 (6)	456 (1)	24	11
7	2‡	797 ± 46 (7)	191 ± 21 (5)	574 ± 59 (7)	440 ± 20 (5)	21	15
4	3	940 ± 115 (6)	273 ± 92 (6)	217 ± 60 (6)	106 ± 22 (6)	29	4
1	3	1306 (1)	335 ± 14 (2)	690 (1)	545 ± 19 (2)	32	15
6	3	1039 ± 159 (3)	318 ± 46 (3)	548 ± 144 (3)	501 ± 46 (3)	27	9
5	3	846 ± 36 (3)	138 ± 23 (4)	228 ± 36 (3)	161 ± 26 (4)	34	9
5	3‡	938 ± 43 (3)	230 ± 106 (4)	337 ± 21 (3)	240 ± 16 (4)	30	11
5	3‡	844 ± 137 (5)	223 ± 92 (3)	359 ± 89 (5)	271 ± 78 (3)	40	17

*Values = mean ± SEM; values in parentheses = No. of determinations.
†Values = % of dose.
‡Uridine given in combination with 5-FU.

TABLE 3.—Effect of continuous and intermittent uridine infusion on body temperature

Patient No.	Schedule	Dose ($g/m^2/hr$)	Highest temperature during treatment (°C)
1	Continuous	2.5	39.3
	Intermittent	2.0	37.4
	Intermittent	3.0	37.8
2	Continuous	1.0	38.6
3	Intermittent	1.0	37.0
4	Intermittent	3.0	37.6
5	Intermittent	3.0	38.2
	Intermittent*	3.0	38.2
	Intermittent*	3.0	37.8
	Intermittent*	3.0	38.0
6	Intermittent	3.0	39.1
7	Intermittent*	2.0	38.0
	Intermittent*	2.0	39.0
	Intermittent*	2.0	38.4

* In combination with 5-FU.

increase is achieved at uridine plasma concentrations of approximately 1 mM (4). The need for long-term uridine exposure is suggested from metabolic studies showing that 5-FU nucleotides are retained in cells and tissues for up to 3 days after drug exposure (10,11).

Our previous study showed that uridine in gram doses could be safely administered to humans as 1-hr infusions. Due to apparent rapid catabolism and elimination of uridine, exposure of the tissues to uridine was limited. Using 1-hr infusions, no indication of rescue from 5-FU toxicity was attained in two patients (9). Notably, the main aim of that study was to investigate the feasibility of uridine administration to humans. In the present paper, we report the results of a further clinical and pharmacokinetic investigation on the administration of high-dose uridine, given as iv infusions over extended periods. The safety of continuous iv infusion of uridine at doses of 1 and 2.5 $g/m^2/hr$ was uncertain due to rapid onset of fever in two patients. It is likely that the shivering observed previously in patients given 1-hr uridine at high doses was a manifestation of an imminent rise in temperature. Using the intermittent infusion schedule, minor to moderate rises in temperature occurred during a number of courses, and were not considered serious enough to discontinue treatment. Of 12 courses of intermittent infusions, only one at 3.0 $g/m^2/hr$ was discontinued because of this side effect. As yet, the cause of fever resulting from prolonged uridine administration is to be elucidated. The presence of pyrogenic lipopolysaccharides is unlikely, as the limulus test on the formulated nucleoside was negative. It cannot be ruled out that uridine itself or a uridine metabolite, through some unknown pharmacologic mechanism, is responsible for this effect on thermoregulation. An argument for the involvement of a metabolite is that increase in body temperature was more pronounced during continuous uridine infusions than during intermittent infusions. It may be that during the treatment-free intervals the fever-causing agent is eliminated to a subtoxic level. The occurrence of fever in uridine-treated patients, however, is not surprising, since uridine was also found to cause fever in rabbits, an animal model known to be very sensitive in predicting pyrogenic effects of drugs. In a recent study, we compared uridine effects on body temperature in rabbits and mice (12). Briefly, uridine given at high doses (3500 mg/kg) had an opposite effect in mice, with body temperature decreasing in some mice by as much as 10°C. Further work is needed to explain these varied temperature effects of uridine in different species.

The other important clinical toxic effect observed was phlebitis. This was seen during both continuous and intermittent infusions, but not in the earlier 1-hr infusion study. Phlebitis has also been reported to occur during prolonged infusion of thymidine (13). This problem could be circumvented by infusion into the central veins.

With continuous infusion of uridine, we demonstrated the possibility to achieve plasma uridine concentrations in the millimolar range, providing long-term exposure of the tissues to elevated uridine concentrations. However, we had to proceed with intermittent administration of uridine due to the risk of fever. With this schedule, we demonstrated that at doses of 2 or 3 $g/m^2/hr$, peak uridine levels of around 1 mM were achieved at the end of the 3-hr infusion periods. During the treatment-free intervals, uridine concentration decreased rapidly, with a mean half-life of 90 mins (range, 60–120), but was still markedly elevated (138–393 μM for 2 and 3 $g/m^2/hr$) at the start of the next infusion, providing repeated periods of relatively high uridine exposure. No appreciable rise in peak uridine levels was observed with successive 3-hr infusions of uridine during the 72-hr administration period, despite the substantial plasma uridine concentration at the end of the 3-hr infusion-free periods. Presumably, uridine clearance proceeded rapidly enough to offset any accumulation effect during the subsequent infusion period. Also consistent with previous findings (9), plasma uracil concentration remained markedly elevated during and in between intermittent uridine infusions, indicating a rapid and saturated catabolism of uridine to uracil. The same amounts of uridine were excreted unchanged in the urine when compared to the 1-hr infusions (mean values, 28% vs 24%). However, larger amounts of uracil appeared in the urine with the prolonged infusion (10.3% vs 3.4%), suggesting saturation of uracil catabolism at these larger uridine doses. The presence of other uridine metabolites, however, was not investigated.

Studies testing the hypothesis that uridine has the potential to rescue patients from 5-FU toxicity are presently being performed. Any pharmacokinetic interaction between 5-FU and uridine was unlikely in the

first two patients studied. At 3 hrs after 5-FU at the start of uridine infusion, plasma concentration of 5-FU was reduced to < 0.1 μM (data not shown). For further studies, it is recommended that uridine be administered using an intermittent infusion schedule at a dose of 2 g/m²/hr (to avoid the risk of fever), 3 hrs after a bolus injection of 5-FU, which alone would produce bone marrow toxicity.

REFERENCES

1. HEIDELBERGER C. Fluorinated pyrimidines and their nucleosides. *In* Antineoplastic and Immunosuppressive Agents. Handbook of Experimental Pharmacology (Sartorelli AC, and Jones DG, eds). Berlin, Springer-Verlag, 1974, part 1, vol 38, pp 193-231.
2. WILKINSON DS, TISTY TD, and HANAS RJ. The inhibition of ribosomal RNA synthesis and maturation in Novikoff hepatoma cells by 5-fluorouracil. Cancer Res 35:3014-3020, 1975.
3. MAJOR PP, EGAN E, HERRICK D, ET AL. 5-Fluorouracil incorporation into DNA of human breast carcinoma cells. Cancer Res 42:3005-3009, 1982.
4. MARTIN DS, STOLFI RL, SPIEGELMAN S, ET AL. High dose 5-fluorouracil with delayed uridine "rescue" in mice. Cancer Res 42:3964-3970, 1982.
5. KLUBES PK, and CERNA I. Use of uridine rescue to enhance the antitumor selectivity of 5-fluorouracil. Cancer Res 43:3182-3196, 1983.
6. KLUBES PK, CERNA I, and MELDON MA. Uridine rescue from the lethal toxicity of 5-fluorouracil in mice. Cancer Chemother Pharmacol 8:17-21, 1982.
7. EVANS RM, LASKIN JD, and KAKALA MT. Assessment of growth limiting events caused by 5-fluorouracil. Cancer Res 40:4113-4122, 1980.
8. SAWYER RC, STOLFI RL, SPIEGELMAN S, ET AL. Effect of uridine on the metabolism of 5-fluorouracil in the $CD8F_1$ murine mammary carcinoma system. Pharm Res 2:69-75, 1984.
9. LEYVA A, VAN GROENINGEN CJ, KRAAL I, ET AL. Phase I and pharmacokinetic studies of high-dose uridine intended for rescue from 5-fluorouracil toxicity. Cancer Res 44:5928-5933, 1984.
10. CHADWICK M, and ROGERS WI. The physiological disposition of 5-fluorouracil in mice bearing solid L1210 lymphocytic leukemia. Cancer Res 32:1045-1056, 1972.
11. WILKINSON DS, and CRUMLEY J. The metabolism of 5-fluorouridine toxicity in Novikoff hepatoma cells. Cancer Res 36:4032-4038, 1976.
12. PETERS GJ, VAN GROENINGEN CJ, LEYVA A, ET AL. Species dependent effect of high-dose uridine on body temperature. Proc Am Assoc Cancer Res 26:370, 1985.
13. SCHORNAGEL JH, LEYVA A, BUESA JM, ET AL. Thymidine prevention of methotrexate toxicity in head and neck cancer. *In* Clinical Pharmacology of Antineoplastic Drugs (Pinedo HM, ed). Elsevier/North Holland, Biomedical Press, 1978, pp 83-95.

Phase II Study of Lonidamine in Patients With Metastatic Renal Cell Carcinoma: A National Cancer Institute of Canada Clinical Trials Group Study[1,2]

Brian H. Weinerman,[3] Elizabeth A. Eisenhauer,[4,*] Jean-Guy Besner,[5] Christopher M. Coppin,[6] David Stewart,[7] and Pierre R. Band[6,8]

The National Cancer Institute of Canada Clinical Trials Group conducted a phase II study of lonidamine, given in an escalating oral daily schedule in patients with measurable advanced renal cell carcinoma. Two responses were seen in 25 evaluable patients. Toxicity was mild or moderate in most patients and included myalgia, nausea, vomiting, somnolence, and testicular pain. Lonidamine was not myelosuppressive. This agent had only minimal activity against renal cell carcinoma when given in this oral schedule. [Cancer Treat Rep 70:751–754, 1986]

Lonidamine is a substituted indazole carboxylic acid originally developed as an antispermatogenic agent. Evidence from biochemical and ultrastructural studies suggests that it interferes with mitochondrial function, thereby inhibiting cellular oxygen consumption (1,2). This drug has activity against animal tumor systems (Lewis lung and Sarcoma 180) (3,4) and produces little toxicity in animals (5).

Phase I studies in man have indicated that the dose-limiting toxic effect is myalgia, which appears at a dose level of 450 mg/day orally. This symptom is often improved by the addition of small doses of prednisone and quickly disappears when the drug is discontinued. The mechanism of the muscle pain is not known, but it is not associated with a rise in creatine phosphokinase levels. Other toxic effects, including somnolence, anorexia, nausea, chills, fever, hyperesthesia, testicular pain, and hot flashes, are sometimes seen. Lonidamine is not myelosuppressive (6).

This report summarizes the results of a phase II study of oral lonidamine in patients with renal cell carcinoma.

MATERIALS AND METHODS

Thirty-three patients with advanced renal cell carcinoma from four centers were entered in the study between April 1982 and May 1984. Eligibility requirements included histologically confirmed renal cell carcinoma, the presence of measurable lesions, Eastern Cooperative Oncology Group (ECOG) performance status ⩽ 2, normal hematologic parameters, bilirubin ⩽ 1.5 mg/100 ml, and creatinine ⩽ 1.5 mg/100 ml. Patients may have received no more than one prior treatment regimen. Written informed consent was obtained from all patients.

Treatment Regimen

Lonidamine was given daily orally in two divided doses using an escalating schedule as shown in table 1.

Escalations were carried out as indicated provided toxicity in individual patients was not prohibitive.

Doses were decreased for moderate or severe myalgia, skin reaction, vomiting, and somnolence to reduce these

[1]Received June 12, 1985; revised Dec 20, 1985; accepted Jan 7, 1986.

[2]Supported in part by a grant from the Angelini Francesco Research Institute, Montreal, Quebec, Canada.

[3]Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada.

[4]National Cancer Institute of Canada Clinical Trials Group, Queen's University, Kingston, Ontario.

[5]Faculte de Pharmacie, Universite de Montreal, Quebec, Canada.

[6]Cancer Control Agency of British Columbia, Vancouver, Canada.

[7]Ontario Cancer Treatment and Research Foundation, Ottawa Regional Cancer Centre, Canada.

[8]We thank the following individuals who, in addition to the authors, contributed patients to the study: G. Armitage (Saskatoon Cancer Clinic, University Hospital, Saskatchewan, Canada); E. Bow (Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada); L. Brandes (Manitoba Cancer Treatment and Research Foundation, Canada); J. Jolivet (Hôpital Notre Dame, Montreal, Quebec, Canada); J. Maroun (Ontario Cancer Foundation, Ottawa Clinic, General Hospital Division, Canada); N. Murray (Cancer Control Agency of British Columbia, Vancouver, Canada); E. W. Ramsey (Department of Urology, University of Manitoba, Winnipeg, Canada); H. Schapper (Manitoba Cancer Treatment and Research Foundation); L. Stolbach (Ontario Cancer Foundation, Ottawa Clinic, Civic Hospital Division); and D. Walde (Algoma District Medical Group, Sault Ste Marie, Ontario, Canada).

[*]*Reprint requests to:* Dr. Elizabeth A. Eisenhauer, National Cancer Institute of Canada Investigational New Drug Program, Queen's University, 82–84 Barrie St, Kingston, Ontario K7L 3N6, Canada.

TABLE 1.—Lonidamine dose and schedule

Day(s)	Dose (mg/m^2)	Route
1	60	Oral
2	120	Oral
3-14	180	Oral
14-28	270	Oral
28-42	360	Oral
42-	450 (maximum)	Oral

symptoms to a grade I (mild) level. In addition, prednisone (5 mg orally twice daily) could be added in instances of moderate or severe myalgia since this had been shown to provide symptomatic relief in a phase I study.

Patients were considered evaluable for response after a minimum of 4 weeks of therapy and evaluable for toxicity from initiation of treatment.

Standard criteria of response were used: complete response was defined as the disappearance of all detectable disease for at least 4 weeks; partial response was defined as a minimum of 50% decrease in the sum of the products of the diameters of measurable disease (with the appearance of no new lesions) for at least 4 weeks; stable disease was defined as a $< 50\%$ decrease or 25% increase in tumor size lasting at least 8 weeks; and progressive disease was defined as a $\geq 25\%$ increase in tumor size and/or the appearance of new lesions.

RESULTS

Thirty-three patients were entered and 31 were eligible. Twenty-eight patients were evaluable for toxicity and 25 were evaluable for response. Reasons for the inevaluable status included two patients who were noncompliant and refused to take their treatment, one patient who deteriorated before 1 week of treatment was complete, two patients who went off study for toxicity (or presumed toxicity), and one patient who started radiation therapy to the only measurable site of disease at the time of going on study.

Patient characteristics are described in table 2. The median age was 57 years, with a median ECOG performance status of 1. The major disease site was lung (17 patients). Twenty-three patients were previously untreated.

Response Data

As shown in table 3, two of the 25 evaluable patients achieved partial response of 22 and 51 weeks, respectively. Sites of response were lung in one patient and nodal disease in the other. Nine patients had stable disease, with a median duration of 19 weeks, and 14 progressed on therapy.

Only 16 of the 31 eligible patients had lonidamine escalated to the planned maximum dose of 450 mg/m^2 by 6 weeks. Most of those who did not (12 patients) went off study for a variety of reasons [progressive disease (five patients), noncompliant (two), toxicity (three), and disease complications (two)] before the 6-week period was complete. Of note, however, is the fact that both patients who achieved partial response were documented to have done so by Week 4.

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 57 (23-73)	
Sex	
Male	23
Female	8
ECOG performance status	
0	9
1	11
2	11
Prior therapy	
None	23
1 agent	8
Major disease site	
Lung	17
Bone	4
Liver	2
Kidney (or retroperitoneum)	8
Previous nephrectomy	19

Toxicity

The overall toxicity of lonidamine was mild and is shown in table 4. There was no evidence of myelosuppression. Myalgia was the predominant toxic effect and was found in 24 patients. Nine of 14 patients who were treated for their myalgia with prednisone (10 mg daily) had an improvement in this symptom. Occasionally, patients experienced mild or moderate nausea and vomiting. Of some interest was the symptom of mild or moderate testicular pain experienced by ten of the 21 male patients entered in the study. Three men had measurement of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) after a minimum of 4 weeks of treatment with lonidamine and all three showed the same pattern of a fall in testosterone to castration levels and an elevation of LH and FSH. In one patient no pretreatment hormone values are available, but these limited observations would suggest that lonidamine acts not only to inhibit spermatogenesis but also produces a decrease in testosterone production by direct effects on the

TABLE 3.—Tumor response

Response	No. of patients	Duration (wks)
Complete	0	
Partial	2	22, 37+
Stable disease	9	Median, 19 (range, 10-40)
Progressive disease	14	

TABLE 4.—Toxicity*

	Toxicity			
	Mild	Moderate	Severe	Total
Somnolence	6	3		9
Myalgia†	15	7	2	24
Skin hypersensitivity	2	1		3
Nausea and vomiting	8	5		13
Diarrhea	4	1		5
Fever and chills	3			3
Tinnitus	1	2		3
Testicular pain‡	5	5		10
Headache	1			1
Hepatotoxicity	1	1		2
Hot flashes§	2	1		3

*Twenty-eight evaluable patients (21 male and 7 female). Values = No. of patients ever to experience toxicity.

†Fourteen of 24 patients with myalgia were treated with prednisone and 9 of 14 improved without a dose reduction of lonidamine.

‡Ten of 21 patients had testicular pain. Some reported decreased libido and impotence. 3 patients who had hormone assessment showed the same pattern on lonidamine: decreased testosterone, increased LH, and increased FSH.

§All male.

testis. A fall in libido and hot flashes while receiving lonidamine were also remarked on by several patients. Three patients were taken off study for reasons of toxicity, one because of a (reversible) rise in liver enzymes and two because of systemic malaise.

Blood Sampling

When possible, blood was drawn 12 hours after the evening dose and 90 minutes after the morning dose of lonidamine to measure lonidamine plasma concentration.

Following centrifugation, the plasma was separated, frozen, and sent to a central laboratory for analysis.

Lonidamine was assayed using a specific high-performance liquid chromatography procedure developed for phase I trials of the drug (7,8).

Plasma Levels of Lonidamine

Twelve patients had blood samples assayed for lonidamine levels but data for all four dose levels were complete in only two.

The mean C_{min} and C_{max} of lonidamine in plasma determined on at least five patients at each dosage level are shown in table 5. The numbers in parentheses represent individual plasma concentrations measured in the two patients for whom data were available at all dose levels. There was considerable variation in the plasma levels of lonidamine measured in different patients at the same dose level. In the first patient, C_{max} and C_{min} rose progressively with increasing dose, but in the second this pattern was not seen.

This sort of variability was seen as well in an earlier oral phase I pharmacokinetic study (8). In that study there was a tenfold variability in the amount of drug absorbed when measured as area under the curve, while the amount excreted as glucuronide in the urine remained fairly constant at approximately 70% of the administered dose (8).

The observations in this phase II study are consistent with the phase I data previously reported and indicate that plasma levels of lonidamine after oral absorption may be low and are variable from patient to patient.

CONCLUSION

Lonidamine appears to have only minimal activity in metastatic renal cell carcinoma, with an 8% partial remission rate. The 95% upper confidence limit of this observed response rate is 23%. Muscular symptoms, testicular pain, and flushing were seen but were relatively nontroublesome side effects. No myelosuppression was seen. The results of this study do not support an active role for this drug as single-agent oral therapy in renal cell carcinoma.

TABLE 5.—Mean plasma concentration (mg/L) of lonidamine at steady state

Daily dose (mg/m^2)	12-hr trough (mean C_{min} ± SD)	1.5-hr (mean C_{max} ± SD)
180	2.1 ± 0.9 (3.6, 0.5)*	12.9 ± 5.5 (14.5, 4.1)*
270	3.5 ± 1.8 (5.8, 0.7)*	14.2 ± 5.9 (20, 5.3)*
360	3.6 ± 2.0 (—, 1.6)*	15.2 ± 6.2 (—, 11.7)*
450	4.5 ± 2.3 (7.7, 2.7)*	21.7 ± 9.4 (35.7, 2.8)*

*Individual plasma concentrations in 2 patients.

REFERENCES

1. FLORIDI A, DEMARTINO C, MARCANTE ML, ET AL. Morphological and biochemical modifications of rat germ cell mitochondria induced by new antispermatogenic compounds. Studies in vivo and in vitro. Exp Mol Pathol 35:314-331, 1981.
2. FLORIDI A, PAGGI MG, D'ATRI S, ET AL. Effect of lonidamine on the energy metabolism of Ehrlich ascites tumor cells. Cancer Res 41:4661-4666, 1981.
3. CAPUTO A. Preliminary results in some tumor systems with indazole-carboxylic acids. Chemotherapy 27(suppl 2):107-120, 1981.
4. SILVESTRINI B, HAHN G, CIOLI V, ET AL. Effects of lonidamine alone or combined with hyperthermia in some experimental cell and tumour systems. Br J Cancer 47:221-231, 1983.
5. HEYWOOD R, JAMES RW, SCORZA BARCELLONA P, ET AL. Toxicological studies on 1-substituted indazole-3-carboxylic acids. Chemotherapy 37(suppl 2):91-97, 1981.
6. BAND P, DESCHAMPS M, BESNER JG, ET AL. Phase I toxicologic study of lonidamine in cancer patients. Oncology 41(suppl 1):53-56, 1984.
7. LECLAIRE R, BESNER JG, BAND P, ET AL. High-performance liquid chromatography of lonidamine in human plasma. J Chromatogr 277:427-432, 1983.
8. BESNER JG, LECLAIRE R, BAND P, ET AL. Pharmacokinetics of lonidamine after oral administration in cancer patients. Oncology 41(suppl 1):48-52, 1984.

Ip Chemotherapy Employing a Regimen of Cisplatin, Cytarabine, and Bleomycin[1,2]

Maurie Markman,* Stephen Cleary, William Lucas, Regis Weiss, and Stephen B. Howell[3]

Thirty-seven patients with refractory ovarian carcinoma or other malignancies principally confined to the peritoneal cavity were treated with a combination ip chemotherapy regimen consisting of cisplatin (100 or 200 mg/m^2), cytarabine (1200 mg/m^2), and bleomycin (15 or 2 U/m^2) repeated at 28-day intervals. Sodium thiosulfate was simultaneously administered iv to protect against cisplatin-induced nephrotoxicity. While only one of 18 patients (6%) with bulky residual ovarian carcinoma experienced a partial remission, two of six evaluable patients with minimal residual disease experienced surgically defined complete remissions. Local abdominal pain was often severe with the higher dose of bleomycin and was occasionally a problem with the lower dose schedule. Fever was common (> 70% of courses), but was reduced in degree by pretreatment with steroids. We conclude that combination ip chemotherapy with this three-drug regimen can result in objective tumor regressions and surgically defined complete remissions in patients with minimal residual ovarian carcinoma who have failed to achieve a complete remission following cisplatin-based iv chemotherapy. [Cancer Treat Rep 70:755–760, 1986]

The ip administration of chemotherapeutic agents as therapy for malignancies principally confined to the abdominal cavity has sound theoretical support (1). Investigators at several centers have demonstrated a major pharmacokinetic advantage for exposure of the peritoneal cavity to several antineoplastic agents [cisplatin (2–4), melphalan (5), mitomycin (6), methotrexate (7,8), 5-FU (9), doxorubicin (10), and cytarabine (11)] compared to exposure of the systemic circulation when the drugs are delivered by the ip route. In addition, surgically and clinically defined responses in patients with malignancies refractory to systemically delivered chemotherapy have been observed following ip treatment (12–14).

A natural extension of single-agent chemotherapy regimens is the administration of a combination of antineoplastic drugs ip. In several malignancies, including ovarian carcinoma, a higher response rate and improved survival have been demonstrated with the use of combination chemotherapy compared to the treatment with single agents (15). We have previously reported our experiences with the ip administration of cisplatin, cytarabine, and doxorubicin (12), and with cisplatin and a higher concentration of cytarabine (13). In both programs, definite clinical activity was demonstrated in patients with refractory ovarian carcinoma. Unfortunately, the regimen which included doxorubicin caused an excessive amount of abdominal discomfort (12).

It is the purpose of our program to develop a safe and effective ip treatment regimen for ovarian carcinoma. In this report, we describe the results of an ip treatment program employing a combination regimen of cisplatin, cytarabine, and bleomycin in a group of 37 patients with refractory ovarian carcinoma or other neoplasms principally confined to the peritoneal cavity.

MATERIALS AND METHODS

Patient Characteristics

Thirty-seven patients (34 female, three male) with a histologically confirmed diagnosis of either ovarian carcinoma or other malignancy whose principal disease

[1]Received Aug 14, 1985; revised Jan 7, 1986; accepted Jan 21, 1986.

[2]Supported in part by Public Health Service grants CA-23100 and CA-35309 (National Cancer Institute) and RR-00827 (Division of Research Resources) from the National Institutes of Health, Department of Health and Human Services, and by the Clayton Foundation for Research, California Division. S. B. Howell is a Clayton Foundation Investigator and M. Markman is an American Cancer Society Junior Faculty Clinical Fellow.

[3]Departments of Medicine and Obstetrics and Gynecology, the Cancer Center; and the General Clinical Research Center, University of California at San Diego, La Jolla.

**Reprint requests to:* Maurie Markman, MD, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

manifestations involved the peritoneal cavity were treated on this experimental ip chemotherapy trial. The median age of the population treated was 58 years (range, 29-74). Thirty patients had ovarian carcinoma, while seven had a mixture of other solid tumors (endometrial, colorectal, sarcoma, and adenocarcinoma of unknown primary). Twenty patients had an Eastern Cooperative Oncology Group performance status of 1, 15 had a performance status of 2, and two had a performance status of 3.

All patients with ovarian carcinoma had failed standard systemically administered chemotherapy, with all but two patients (7%) having previously received iv cisplatin. Twenty patients (71%) with ovarian carcinoma had previously responded to iv cisplatin (complete and partial remissions), but subsequently relapsed. One patient with endometrial carcinoma had been given systemically delivered cisplatin prior to entry onto this trial. While all patients with refractory ovarian carcinoma had documented residual disease before the institution of the experimental chemotherapy program, six patients (20%) had disease defined only surgically, and failing to undergo a subsequent exploratory laparotomy, are only evaluable for toxicity and not efficacy of the treatment program.

As this was a trial designed to evaluate the toxicity as well as efficacy of the three-drug combination, patients with bulky disease were not excluded from the treatment program even though it was recognized that the chance for therapeutic benefit being observed in this clinical situation was quite small. Patients were only excluded from this program if they did not meet entry criteria (histologically confirmed malignancy whose principal disease manifestations involved the peritoneal cavity, serum creatinine $\leq$ 1.5 mg/100 ml, wbc count $\geq$ 3000/mm^3, platelet count $\geq$ 100,000/mm^3, serum bilirubin $\leq$ 3.0 mg/100 ml), if they had medical contraindications to catheter placement, or if the surgeon felt it was not possible to safely insert a peritoneal catheter because of extensive tumor or adhesions.

Eighteen patients with refractory ovarian carcinoma had disease where at least one tumor mass was $\geq$ 2 cm in diameter (bulky residual disease), while 12 patients had disease where the largest tumor nodule was < 2 cm in diameter (minimal residual disease).

Method of Drug Delivery

All patients participating in this treatment program had a semipermanent indwelling peritoneal dialysis catheter (Tenckhoff type) surgically placed and attached to a subcutaneous port (Port-A-Cath, Pharmacia Nu-Tech, Piscataway, NJ) prior to the institution of the ip chemotherapy program (16). The port was placed under the skin and over a rib to allow for easy access to the peritoneal cavity. In patients with bulky intra-abdominal disease, an ultrasound examination was performed to assist in catheter placement. The ports and catheters were only entered at the time of treatment, to drain ascites, or to obtain peritoneal cavity cytologies. Following catheter manipulation, which was always done using strict aseptic technique, the port and catheter were flushed with heparin. In one patient, the catheter slipped out of the peritoneal cavity and replacement was required. In a second patient, at the time of his fourth treatment, we were unable to deliver the treatment volume because of extensive adhesions which caused the catheter to become tightly adherent to the anterior abdominal wall. In approximately 40% of patients at some time during their treatment course we were able to remove only a small amount or no fluid through the surgically implanted catheter ("one-way valve"). This appeared to be secondary to the formation of a fibrous sheath around the catheter.

The adequacy of distribution of the treatment volume was not routinely evaluated in our patient population. It had previously been demonstrated that approximately 70% of patients, even those with bulky intra-abdominal disease, are able to achieve adequate drug distribution if large treatment volumes (2 L) are utilized (2,17).

Treatment Program

Approximately 1 week after catheter placement, patients were admitted to the hospital and begun on iv hydration (2 L of normal saline over the 12 hours prior to chemotherapy instillation). With the initiation of drug instillation into the peritoneal cavity, sodium thiosulfate was delivered both as an iv bolus (4 g/m^2 in 250 ml of sterile water) and as a continuous infusion (12 g/m^2 in 1 L of sterile water over 6 hours) in an effort to prevent cisplatin-induced renal dysfunction. The three chemotherapeutic drugs were mixed together and delivered through the port as rapidly as possible in 2 L of normal saline. The usual duration of the ip instillation was 30-45 minutes. As much of the remaining fluid as possible was removed 4 hours after the completion of the chemotherapy instillation.

Therapy was repeated at 28-day intervals. Patients received at least two courses of treatment unless there was unacceptable toxicity, obvious disease progression, or clinical deterioration. In patients demonstrating a response to therapy (subjective or objective) or in those with unevaluable disease, a total of six courses of treatment were administered. Therapy was stopped in any patient with excessive toxicity or disease progression. Patients with ovarian carcinoma in a clinical complete remission at the completion of therapy were offered the option of an exploratory laparotomy to further define the status of disease.

Chemotherapy Dosages

The dose of cisplatin was either 100 or 200 mg/m^2.

Patients initially received the 200-mg/m^2 dose unless they had previously experienced neurotoxicity, nephrotoxicity (serum creatinine rise to > 2.0 mg/100 ml), or severe bone marrow depression with prior therapy. Patients with these complications of previous treatment received cisplatin at a dose of 100 mg/m^2, as did those patients experiencing severe emesis, nephrotoxicity, neurotoxicity, or significant myelosuppression with the initial course of the ip treatment program. Cytarabine was delivered at a dose of 1200 mg/m^2. Bleomycin was initially administered at a dose of 15 U/m^2 but, as will be discussed later, the dose was subsequently decreased to 2 U/m^2.

In Vitro Evaluation of Three-Drug Combination

The stability of the three-drug treatment regimen was evaluated in vitro before the initiation of the clinical trial. A solution of cisplatin (0.2 mg/ml), cytarabine (1.2 mg/ml), and bleomycin (0.12 U/ml) was prepared in 0.9% sodium chloride. The solution was maintained at room temperature for 24 hours (standard laboratory lighting). During this period of time, there was no change in pH or color and there was no evidence of precipitate formation. The concentrations of the three drugs were analyzed to determine if there was any loss of active drug following mixing. Cisplatin and cytarabine were measured by liquid chromatography, while bleomycin levels were determined by radioimmune assay (2,18,19). The cisplatin assay measures only that fraction of the drug which is still reactive with a nucleophilic site (20). There was no detectable loss of cisplatin over the 24-hour period, while cytarabine levels increased by 10% and bleomycin levels decreased by 9%.

RESULTS

A total of 120 courses of therapy were administered to the 37 patients participating in this trial. Cisplatin was delivered at a dose of 100 mg/m^2 in 81 courses, while patients received this drug at a dose of 200 mg/m^2 during 39 courses. Bleomycin was delivered at a dose of 15 U/m^2 in 13 courses, while in the remaining courses the drug was delivered at 2 U/m^2. The median number of courses per patient was three (range, one to six), with the median ip dose of cisplatin administered during this trial being 400 mg/m^2 (range, 100–1200).

Toxicity

Systemic toxicity was, in general, quite mild except for cisplatin-induced emesis which was universal and occasionally quite severe at the 200-mg/m^2 dose of cisplatin, despite an aggressive antiemetic regimen. However, no patient was forced to withdraw from the study because of this complication of treatment.

Seven courses (7%) were associated with a serum creatinine rise to ⩾ 1.5 mg/100 ml (upper limit of normal in our laboratory), with only a single course of treatment being complicated by a serum creatinine rise to ⩾ 2.0 mg/100 ml (2.1 mg/100 ml). In all cases, the serum creatinine returned to baseline within 1 month of therapy. Eleven courses (14%) with cisplatin administered at 100 mg/m^2 were associated with wbc count nadirs of < 3000/mm^3, while following eight courses (21%) with this drug at 200 mg/m^2, the wbc count fell to < 3000/mm^3. However, following only three courses did the wbc count drop to < 2000/mm^3 (1900, 1700, and 1500/mm^3). The platelet count dropped to < 100,000/mm^3 after a single treatment cycle (95,000/mm^3).

Local toxicity was a greater problem. More than fifty percent of patients receiving the 15-U/m^2 dose of bleomycin experienced an unacceptable degree of abdominal pain (lasting > 72 hours or requiring narcotic analgesia). With the 2-U/m^2 dose, abdominal pain was less severe, but several patients who also received cisplatin at a dose of 200 mg/m^2 were quite uncomfortable because of the combination of emesis and pain. Seventy percent of courses with bleomycin administered at the high-dose level were associated with a temperature elevation to > 101.5°F. Temperatures to this range developed following 30% of courses with the 2-U/m^2 dose. However, when steroids were added to the treatment regimen, only 5% of courses were complicated by temperature rises to > 101.5°F.

Two episodes of bacterial peritonitis developed during this trial. In one case, the catheter port eroded through the skin. Unfortunately, this development was not brought to medical attention until the patient developed a *Staphylococcus aureus* peritonitis which was ultimately fatal. In addition, two patients developed partial small bowel obstructions. In one case this was felt to be secondary to the chemotherapy program, as no tumor was found at surgery.

Response to Therapy of Patients With Refractory Ovarian Carcinoma

Of the 30 patients with refractory ovarian carcinoma, 18 (60%) had bulky residual intra-abdominal disease, while 12 had minimal residual disease. Only one patient with bulky disease (6%) experienced a decrease in the size of a mass lesion (partial remission of an intra-abdominal mass), with a second patient having disappearance of ascites for 4 months. Two patients with minimal residual disease experienced complete responses which were defined at laparotomy (response durations of 9+ and 13+ months), with an additional patient having had complete disappearance of ascites but continuing to have positive cytologies. A final patient with refractory ovarian carcinoma and minimal residual disease had clearing of positive cytologies but

elected not to undergo a repeat laparotomy to further define the status of disease. Of the six patients demonstrating evidence of a clinical response to the experimental ip chemotherapy program, all had previously responded to cisplatin-based systemic chemotherapy.

The overall median survival for the patients with refractory ovarian carcinoma treated on this trial has not been reached, but will exceed 7 months (range, 1-17+). To date, seven patients (23%) have died, six with bulky disease and one with minimal residual disease. The six patients with nonevaluable disease remain alive and clinically disease-free, with a median follow-up of 8 months (range, 6-15). Whether any of these patients or those with surgically defined complete remissions are "cured" of their disease must await long-term follow-up.

Response of Patients With Non-ovarian Cancer

Of the six patients with non-ovarian cancer treated on this experimental ip chemotherapy trial, two demonstrated evidence of a response to treatment. The patient with adenocarcinoma of unknown primary presented with abdominal pain, weight loss, and massive ascites and was requiring weekly paracentesis of > 5 L of fluid. He experienced complete disappearance of ascites, a weight gain of 20 lbs, and disappearance of pain which lasted for 10 months. A second patient with endometrial carcinoma experienced disappearance of ascites which persisted for a period of 4 months. There was no change in the size of her bulky intra-abdominal masses. The median duration of survival for this group of patients was 8 months (range, 2-17+).

DISCUSSION

The administration of a combination chemotherapy regimen directly into the peritoneal cavity as therapy for tumors principally confined to this region of the body, such as ovarian carcinoma, has significant theoretical appeal. The delivery of several drugs active against a particular tumor type might increase the efficacy of therapy while at the same time decreasing the opportunity for the development of drug resistance (21,22). In addition, the ip delivery of a combination chemotherapy regimen where the cavity is exposed to higher concentrations of drugs than can be achieved if the agents are delivered systemically optimizes the chances of observing in vivo the beneficial effects of concentration-dependent drug synergy demonstrated in vitro. For example, cisplatin and cytarabine have been shown to be markedly synergistic against a colon carcinoma cell line (LoVo) in a concentration-dependent manner (23). At the highest concentration of cytarabine evaluated in this experimental system (4×10^{-2} M), the cell kill produced by the combination of cisplatin and cytarabine was 1600-fold greater than that produced by cisplatin alone. While this concentration of cytarabine is not achievable in man with iv drug delivery, it is at least approachable during ip therapy because of the liver's ability to deaminate cytarabine to a nontoxic metabolite (uracil arabinoside) prior to its entry into the systemic circulation. In the trial reported here, we have used cytarabine at a concentration of 4×10^{-3} M. This concentration is approximately three logs higher than that achieved at steady-state concentrations in the plasma when cytarabine is administered iv (in acute leukemia) at a standard dose of 100 mg/m^2 by continuous infusion (24).

Bleomycin was added to our ip regimen for several reasons. First, in several malignancies, most notably testicular carcinoma, a cisplatin-bleomycin combination has resulted in impressive clinical activity (25,26). Second, it has recently been demonstrated that potentially important interactions between cisplatin and bleomycin occur at the level of DNA-drug binding (27). Third, a major pharmacokinetic advantage for peritoneal cavity exposure to bleomycin has been shown (C. E. Pfeifle, unpublished data), with only 45% of the ip administered dose being recovered in the systemic circulation or urine (28,29). Finally, activity for single-agent bleomycin against refractory ovarian carcinoma has been demonstrated both in vitro and in vivo (29-31).

While definite clinical activity (including surgically defined complete remissions in refractory ovarian carcinoma) was demonstrated in this trial, the dose of bleomycin administered was limited by excessive abdominal pain. This is similar to our experience with a combination ip regimen which included doxorubicin (12). While most patients were able to tolerate the lower dose of bleomycin (2 U/m^2), abdominal pain remained a problem, and the combination of this factor and cisplatin-induced emesis at the 200-mg/m^2 dose level of this agent would make this a difficult regimen to deliver multiple times as part of the standard treatment program. With the lower dose of cisplatin, the severity of emesis was less, and abdominal discomfort was similarly not as great a problem. Unfortunately, decreasing the concentration of cisplatin potentially interferes with the efficacy of therapy, particularly when thiosulfate is delivered systemically to protect against cisplatin-induced nephrotoxicity (2,32). Whether it will be possible to administer the higher concentrations of cisplatin or bleomycin if the agents were delivered at different times will require further clinical evaluation. In addition, the long-term effects of an ip regimen which employs bleomycin must be evaluated, as the agent is a known effective sclerosing agent when delivered in much higher concentrations into the pleural or peritoneal cavities (33). In fact, in the patients demonstrating only a decrease in ascites in our trial, it is unknown whether the clinical improvement was secondary to a cytotoxic or sclerosing effect of the therapeutic program.

Much work remains to be done in the area of intracavitary chemotherapy in defining optimal drug combinations and concentrations and in addressing the technical problems associated with this approach (drug distribution and delivery, infectious and chemical peritonitis). It is possible that a therapeutic program for a tumor type principally confined to the abdominal cavity which includes simultaneous or sequential systemically and ip administered agents would be superior to standard systemic therapy. It is also conceivable, because of the tremendous pharmacokinetic advantage the ip approach offers, that an all ip delivery program will prove to be a more effective therapeutic program (34). Agents delivered in such a program must be able to enter the systemic circulation in high enough concentrations so as not to interfere with the delivery of drug to tumor by capillary flow. It should be noted that patients with bulky intra-abdominal disease are unlikely to benefit significantly from the ip approach because of the known limited penetration of currently available chemotherapeutic agents. Finally, the ultimate role for ip chemotherapy in treating any tumor type confined to the peritoneal cavity must be defined in carefully designed randomized controlled clinical trials, as it is unknown at present if the ip approach will prove to be more effective than other methods of intensive drug delivery.

REFERENCES

1. DEDRICK RL, MYERS CE, BUNGAY PM, ET AL. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1-9, 1978.
2. HOWELL SB, PFEIFLE CE, WUNG WE, ET AL. Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97:845-851, 1982.
3. CASPER ES, KELSON DP, ALCOCK NW, ET AL. Ip cisplatin in patients with malignant ascites: pharmacokinetic evaluation and comparison with the iv route. Cancer Treat Rep 67:235-238, 1983.
4. PRETORIUS RG, HACKER NF, BEREK JS, ET AL. Pharmacokinetics of ip cisplatin in refractory ovarian carcinoma. Cancer Treat Rep 67:1085-1092, 1983.
5. HOWELL SB, PFEIFLE CE, and OLSHEN RA. Intraperitoneal chemotherapy with melphalan. Ann Intern Med 101:14-18, 1984.
6. ADAMS SC, PATT YZ, and ROSENBLUM MG. Pharmacokinetics of mitomycin C following intraperitoneal administration of mitomycin C and floxuridine for peritoneal carcinomatosis. Proc Am Assoc Cancer Res 25:316, 1984.
7. HOWELL SB, CHU BCF, WUNG WE, ET AL. Long-duration intracavitary infusion of methotrexate with systemic leucovorin protection in patients with malignant effusions. J Clin Invest 67:1161-1170, 1981.
8. JONES RB, COLLINS JM, MYERS CE, ET AL. High-volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41:55-59, 1981.
9. SPEYER JL, COLLINS JM, DEDRICK RL, ET AL. Phase I pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:567-672, 1980.
10. OZOLS RF, YOUNG RC, SPEYER JL, ET AL. Phase I and pharmacological studies of Adriamycin administered intraperitoneally to patients with ovarian cancer. Cancer Res 42:4265-4269, 1982.
11. KING ME, PFEIFLE CE, and HOWELL SB. Intraperitoneal cytosine arabinoside in ovarian carcinoma. J Clin Oncol 2:662-669, 1984.
12. MARKMAN M, HOWELL SB, LUCAS WE, ET AL. Combination intraperitoneal chemotherapy with cisplatin, cytarabine, and doxorubicin for refractory ovarian carcinoma and other malignancies principally confined to the peritoneal cavity. J Clin Oncol 2:1321-1326, 1984.
13. MARKMAN M, HOWELL SB, CLEARY S, ET AL. Intraperitoneal chemotherapy with high dose cisplatin and cytarabine for refractory ovarian carcinoma and other malignancies principally involving the peritoneal cavity. J Clin Oncol 3:925-931, 1985.
14. MCVIE JG, TEN BOKKEL HUININK WW, ET AL. Intraperitoneal chemotherapy in minimal residual ovarian cancer with cisplatin and IV sodium thiosulfate protection. Proc ASCO 4:125, 1985.
15. YOUNG RC, CHABNER BA, HUBBARD SP, ET AL. Advanced ovarian adenocarcinoma: a prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261-1266, 1978.
16. PFEIFLE CE, HOWELL SB, MARKMAN M, ET AL. Totally implantable system for peritoneal access. J Clin Oncol 2:1277-1280, 1984.
17. DUNNICK NR, JONES RB, DOPPMEN JL, ET AL. Intraperitoneal contrast infusion for assessment of intraperitoneal fluid dynamics. AJR 133:221-223, 1979.
18. BREITHAUPT H, and SCHICK J. Determination of cytarabine and uracil arabinoside in human plasma and cerebrospinal fluid by high-performance liquid chromatography. J Chromatogr 225: 99-106, 1981.
19. BROUGHTON A, and STRONG JE. Radioimmunoassay of bleomycin. Cancer Res 36:1418-1421, 1976.
20. ANDREW PA, WUNG WE, and HOWELL SB. A high performance liquid chromatographic assay with improved selectivity for cisplatin and active platinum (II) complexes in plasma ultrafiltrate. Anal Biochem 143:46-56, 1984.
21. GOLDIE JH, and COLDMAN AJ. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727-1733, 1979.
22. GOLDIE JH, COLDMAN AJ, and GUDAUSKAS GA. Rationale for the use of alternating non-cross-resistant chemotherapy. Cancer Treat Rep 66:439-449, 1982.
23. BERGERAT JP, DREWINKO B, CORRY P, ET AL. Synergistic lethal effect of *cis*-dichlorodiammineplatinum and 1-beta-D-arabinofuranosylcytosine. Cancer Res 41:25-30, 1981.
24. SLEVIN ML, PIALL EM, AHERNE GW, ET AL. The pharmacokinetics of cytosine arabinoside in the plasma and cerebrospinal fluid during conventional and high-dose therapy. Med Pediatr Oncol (suppl)1:157-168, 1982.
25. EINHORN LH, and DONOHUE J. *cis*-Diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293-298, 1977.
26. STOTER G, VENDRIK CPJ, STRUYVENBERG A, ET AL. Combination chemotherapy with *cis*-diammine-dichloro-platinum, vinblastine, and bleomycin in advanced testicular non-seminoma. Lancet 1:941-945, 1979.
27. MASCHARAK PK, SUGIURA Y, KUWAHARA J, ET AL. Alteration and activation of sequence-specific cleavage of DNA by bleomycin in the presence of the antitumor drug *cis*-diamminedichloroplatinum(II). Proc Natl Acad Sci USA 80:6795-6798, 1983.
28. ALBERTS DS, CHEN HSG, MAYERSOHN M, ET AL. Bleomycin pharmacokinetics in man. II. Intracavitary administration. Cancer Chemother Pharmacol 2:127-132, 1979.
29. ALBERTS DS, CHEN HSC, CHANG SY, ET AL. The disposition of intraperitoneal bleomycin, melphalan, and vinblastine in cancer patients. Recent Results Cancer Res 74:293-299, 1980.
30. ALBERTS DS, SALMON SE, CHEN HSG, ET AL. In vitro clonogenic assay for predicting response of ovarian cancer to chemotherapy. Lancet 2:340-342, 1980.
31. BLACKLEDGE G, LAWTON F, BUCKLEY H, ET AL. Phase II evaluation of bleomycin in patients with advanced epithelial ovarian cancer. Cancer Treat Rep 68:549-550, 1984.
32. HOWELL SB, and TAETLE R. Effect of sodium thiosulfate on *cis*-dichlorodiammineplatinum(II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat Rep 64:611-616, 1980.

33. OSTROWSKI MJ, and HALSALL GM. Intracavitary bleomycin in the management of malignant effusions: a multicenter study. Cancer Treat Rep 66:1903-1907, 1982.

34. MARKMAN M, and HOWELL SB. Intraperitoneal chemotherapy for ovarian carcinoma. *In* Gyn Oncology (Alberts D, and Surwitt E, eds). Boston, Martinus Nijhoff, 1985, pp 179-212.

Chemoradiotherapy as Initial Management in Patients With Squamous Cell Carcinoma of the Head and Neck[1]

David J. Adelstein,* Vishwa M. Sharan, A. Scott Earle, Ajit C. Shah, Catherine Vlastou, Chandra D. Haria, Susan G. Carter, Catherine Damm, and John D. Hines[2,3]

Thirty-nine previously untreated or minimally treated patients with squamous cell carcinoma of the head and neck were entered onto a chemoradiotherapy protocol employing multiple courses of simultaneous radiation therapy, cisplatin, and a 4-day 5-FU infusion. Thirty-eight patients were evaluable for response and toxicity. Twenty-three patients underwent surgical resection midway through this therapy and 11 (48%) were pathologically free of disease. Thirty-five of the 38 patients (92%) were ultimately rendered disease-free by this combined modality protocol. Thirty-one patients remain disease-free, with a projected 2-year disease-free survival of 74%. Although the treatment-associated mucositis and myelosuppression were significant, this chemoradiotherapeutic approach is promising and merits further study. [Cancer Treat Rep 70:761–767, 1986]

Squamous cell carcinoma of the head and neck is often a locally extensive disease with frequent involvement of cervical lymph nodes. Hematogenous metastases are uncommon (1,2). Traditional therapy, therefore, consists of radical surgery and/or radiation therapy in an effort to achieve local control. Disease behavior suggests that such local therapy, if sufficiently aggressive, should be adequate to achieve cure.

Unfortunately, the cure rate is only 30%–50% (3–6). This reflects, in part, the tendency for patients with this neoplasm to present at an advanced stage (3,6,7). Even radical surgical procedures have only limited success in such patients, and the surgery often results in major disability and disfigurement. Many patients are beyond hope of resection at the time of presentation and, as a result, are given radiation therapy. Although radiation to this region is tolerable even in very large doses, the results are dismal with locally advanced tumors (4,5,8). The cure rates for patients with stage IV disease average < 15% (5,8,9).

The role of chemotherapy in the management of this tumor remains to be defined (3,7,10). Many different agents and combinations have demonstrated response rates of 50%–100% in previously untreated patients (3,6,7,10–16). Unfortunately, these responses to chemotherapy alone tend to be partial and short-lived (3,6,7,10,11,13,14,16). Even in patients achieving complete response, the need remains for definitive surgery and/or radiation therapy (16). The possibility has been raised that initial (neoadjuvant) chemotherapy might be successful both in down-staging patients prior to definitive therapy and in improving long-term survival (3,9,10, 14–16). Although there are numerous enthusiastic but uncontrolled neoadjuvant chemotherapy studies (6,7,12–16), the results of controlled trials have thus far been disappointing (9,17–19).

The radiosensitizing ability of chemotherapeutic agents such as 5-FU and cisplatin has therapeutic promise. A preoperative dose of radiation considered ineffective when given alone could theoretically be of much greater value when given with simultaneous radiosensitizing chemotherapy. Significant down-staging might be possible with limited radiation, thus avoiding the complications of surgery in a heavily irradiated field.

Further exploration of the value of this approach seems warranted in squamous cell carcinoma of the head and neck. In addition to their clinically active radiosensitizing ability, 5-FU and cisplatin are highly active in combination in this disease, even without radiation (15). We report our preliminary results in 39 patients with squamous cell carcinoma of the head and

[1]Received July 31, 1985; revised Dec 30, 1985; accepted Jan 31, 1986.

[2]Departments of Medicine (*D. J. Adelstein, S. G. Carter,* and *J. D. Hines*), Radiology (*V. M. Sharan*), Surgery (*A.S. Earle, A. C. Shah, C. Vlastou,* and *C. D. Haria*), and Nursing (*C. Damm*), Cleveland Metropolitan General Hospital, Case Western Reserve University, OH.

[3]We thank Anne L. LaGania for her secretarial assistance, and the house staff and nurses of Cleveland Metropolitan General Hospital for their patient care.

Reprint requests to: David J. Adelstein, MD, Division of Hematology/Oncology, Department of Medicine, Cleveland Metropolitan General Hospital, 3395 Scranton Rd, Cleveland, OH 44109.

neck treated simultaneously with these agents and radiation therapy.

MATERIALS AND METHODS

Patients with histologically documented, measurable squamous cell carcinoma of the head and neck region, without prior treatment or with minimal prior surgery alone, were eligible for this study. Pretreatment staging evaluation included a medical history, examination under anesthesia, panendoscopy, barium swallow, computerized tomographic scan of the involved region, and chest x-ray. Radionuclide scans and other staging procedures were obtained only if clinically indicated. A cbc, urine analysis, ECG, and serum chemistry tests including urea nitrogen, creatinine, calcium, phosphorus, alkaline phosphatase, SGOT, SGPT, albumin, total protein, bilirubin, and uric acid were performed on all patients. All patients underwent a pretreatment dental evaluation with appropriate care.

Patients were staged according to the TNM system of the American Joint Committee on Cancer (20). Patients with any tumor size (T) or nodal status (N) were eligible. Patients with metastatic disease (M_1) were ineligible. Patients with a serum creatinine > 2.0 mg/dl, a serum bilirubin > 2.5 mg/dl, or an unexplained abnormal pretreatment hemogram were excluded. No age restrictions were placed.

This study was approved by the Cleveland Metropolitan General Hospital Committee on Investigation in Humans. Written informed consent was obtained from all patients before beginning therapy. Patient management was provided by a multidisciplinary team including medical, radiation, and nurse oncologists, otolaryngologists, and plastic and oral surgeons.

Treatment was initiated with both radiation therapy and chemotherapy (fig 1). Patients were given 3000 rads of external-beam radiation therapy in 15 daily fractions over 3 weeks to the entire tumor and node-bearing region. Radiation was generated by either a cobalt-60 source or a 6-MeV linear accelerator, and was administered to midplane without beam modifiers. Concurrent with radiation, patients were given 5-FU (1000 mg/m^2/day for the first 4 days as a continuous iv infusion) and cisplatin (75 mg/m^2 iv on Day 1). The cisplatin was given after vigorous iv hydration with furosemide and mannitol diuresis. Antiemetics were given to all patients as necessary. Between the fifth and seventh weeks, a second identical course of chemotherapy was given but without radiation therapy.

Between 8 and 12 weeks after the initiation of treatment, patients were evaluated for surgery. Definitive surgery was performed unless the patient refused or was considered inoperable. Operability was defined by the ability to obtain tumor-free margins and a functional reconstruction. Midway during this study, surgery became optional for those patients with limited disease at presentation who had achieved complete clinical remissions after preoperative therapy. Inoperability prior to chemoradiotherapy did not affect the decisions made after initial treatment concerning operability or the extent of surgery needed. Surgery consisted of a composite resection of the residual primary tumor with immediate reconstruction, if possible, and a functional or radical neck dissection.

All patients, whether undergoing surgery or not, were then offered postoperative chemoradiotherapy identical to that given preoperatively. An additional 3000 rads of radiation therapy in 15 daily fractions was given concurrent with a third course of 5-FU and cisplatin. The last 1000–1500 rads of this radiation was given to a more limited tumor-encompassing and spinal cord-sparing port. A fourth course of chemotherapy without radiation therapy completed the planned treatment. A total of 6000 rads of radiation therapy and four courses of chemotherapy would, therefore, have been given to patients completing all therapy.

Chemotherapy dosage modifications were not made, irrespective of nadir blood counts or duration of myelosuppression. Chemotherapy courses were delayed up to 3 weeks to allow for full bone marrow and mucosal recovery. If additional delay was considered necessary, the subsequent chemotherapy course was deleted in entirety. Permanent deterioration in renal function was considered a criterion for discontinuation of cisplatin.

Patients were evaluated for clinical response after the first two courses of chemotherapy and the first 3000 rads of radiation. Response was determined by repeating all of the relevant staging procedures. Pathologic confirmation of the clinical response was obtained in those patients undergoing surgery. Clinical response was again evaluated after the completion of all ther-

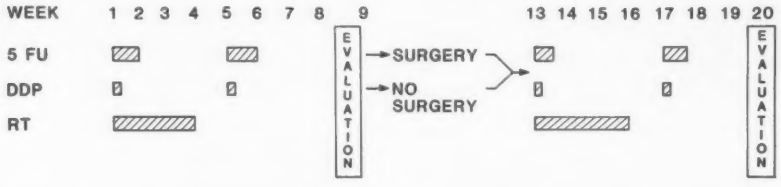


FIGURE 1.—Treatment schema: 5-FU—1000 mg/m^2/day iv continuous infusion for 4 days; cisplatin (DDP)—75 mg/m^2 iv on Day 1; radiation therapy (RT)—3000 rads in 15 fractions over 3 wks.

apy. Patients were subsequently seen every 2 months and clinically assessed for recurrence, with repetition of radiographic procedures only if indicated.

Complete response was defined as the complete disappearance of all radiographic, clinical, and when applicable, pathologic evidence of disease. Partial response was defined as any response less than complete, but with a > 50% reduction in the sum of the products of the crossed diameters of all measurable lesions. Patients with any lesser degree of tumor shrinkage were considered nonresponders. Progressive disease was defined as a > 25% increase in the sum of the products of the crossed diameters of all measurable lesions or the appearance of any locally recurrent or metastatic disease. Disease-free survival time was calculated from the date chemotherapy was initiated.

Disease-free survival curves were constructed using the Kaplan-Meier method. Statistical data were calculated by the chi-squared distribution and the Breslow modification of the generalized Wilcoxon test.

RESULTS

Between February 1983 and June 1985, 39 consecutive eligible patients with squamous cell carcinoma of the head and neck were seen at Cleveland Metropolitan General Hospital, were entered on this treatment protocol, and received sufficient therapy to be considered evaluable. There was a single major protocol violation in a patient who received only 2000 rads of preoperative radiation and experienced a 2-month delay before surgery. No unusual toxicity occurred and although this patient remains alive and disease-free, she has been excluded from all analysis. Four patients had undergone minimal prior surgery. The others were previously untreated. No patients had received radiation therapy or chemotherapy.

The clinical characteristics of the 38 evaluable patients are depicted in table 1. Sex, race, and age distribution are typical for this disease. Five patients were > 70 years of age. All patients had an Eastern Cooperative Oncology Group performance status of 0-1. Thirty-four of the 38 patients had either stage III or IV disease, as is common in an unselected series of patients with head and neck malignancy. Extensive local disease made it difficult to identify more precisely the exact site of tumor origin.

Using the definitions of the American Joint Committee on Cancer, tumor extent (T) and nodal status (N) are given in table 2. In eight patients, a second malignancy had been diagnosed. This included one patient with a synchronous and two patients with metachronous squamous cell cancers of the head and neck. There was one patient with a synchronous and one with a metachronous non-small cell lung cancer, and there were single patients with a history of carcinoma of the cervix, carcinoma of the colon, and malignant melanoma.

TABLE 1.—Patient (N = 38) and tumor (N = 39*) characteristics

Characteristic	No.
Sex	
Male	25
Female	13
Race	
Black	10
White	28
Median age in yrs (range): 57 (32-77)	
Site	
Oral cavity	17*
Oropharynx	13*
Hypopharynx	4
Larynx	5
Histology (all squamous cell)	
Well-differentiated	25
Moderately well-differentiated	9*
Poorly differentiated	5
Stage	
I	1
II	4*
III	15
IV	19

*Includes 1 patient with 2 synchronous stage II tumors.

The toxicity associated with this chemoradiotherapy was considerable (table 3), but there were no toxic deaths. Life-threatening myelosuppression occurred in 16 patients (42%) and was associated with fever, requiring hospitalization in seven (18%). Nephrotoxicity, defined as a > 50% increase in serum creatinine, was noted in 34% but was completely reversible in all patients. Mucositis occurred in all but three patients and resulted in an average weight loss of 7.6 kg (range, 0-23.3), or 11.7% of initial body weight. This mucositis was severe enough to require hospitalization in eight patients (21%). Although vomiting occurred in 35 of 38 patients (92%), it was unresponsive to medical management in only two and required further hospitalization in only a single patient.

One patient, a 32-year-old male with no previous cardiac history, experienced 5-FU-associated cardiotoxicity with transient chest pain, ischemic ECG changes, and global cardiac dysfunction on echocardiography. All abnormalities reverted to baseline within 3 weeks of his

TABLE 2.—Tumor (T) and nodal (N) extent (N = 39*)

	N_0	N_1	N_2	N_3
T_X				2†
T_1	1			1
T_2	4	5	2	
T_3	6	4		1
T_4	6	2	2	3

*Includes 1 patient with 2 synchronous T_2N_0 tumors.
†Nodal recurrence after limited excision of the primary tumor.

TABLE 3.—Chemoradiotherapy toxic effects (N = 38)

Toxic effect	No. of patients (%)
Mucositis	35 (92)
Nausea, vomiting	35 (92)
Myelosuppression	
Granulocyte count < 1000/mm^3	16 (42)
With fever	7 (18)
Platelet count < 50,000/mm^3	1 (3)
Nephrotoxicity (transient)	13 (34)
Cardiac toxicity	1 (3)
Postural hypotension	1 (3)

chemotherapy. The chest pain and ECG changes recurred and again resolved during his postoperative treatment. Another patient, a 76-year-old male, experienced profound but temporary postural hypotension within 2 weeks after each course of chemotherapy. This did not recur when 5-FU was given without cisplatin, and was attributed to the autonomic neuropathy described with cisplatin (21).

Not all of the chemotherapy courses could be given. Only 31 of the 38 patients received their second course of chemotherapy because of the protracted time needed for recovery from treatment-associated toxicity. Seven patients never received postoperative chemoradiotherapy. In two patients, this resulted from a delay in surgical healing, while five patients refused further therapy. Ten additional patients did not receive the fourth chemotherapy course due to delayed recovery from toxicity. Only 16 of the 38 patients received all four courses of chemotherapy. Small weekly doses of methotrexate (20 mg/m^2) were given during radiation therapy to the first two patients treated on this study. Methotrexate was not given to any other patients because of our concern about the mucositis.

Achievement and maintenance of disease-free status (complete response) are depicted in table 4. All patients were at least partial responders and 20 of the 38 patients (53%) achieved either a clinical or pathological complete response at the time of their first evaluation.

Twenty-three patients subsequently underwent surgery and 21 of these 23 were considered free of disease after the surgery. Two patients had incomplete resections. Both died before institution of postoperative therapy, one from disease progression and one from an unrelated cause. Three patients in the surgical group underwent elective lymph node dissection without resection of the primary tumor when no clinical evidence of the primary lesion could be found at the time of surgery.

Fifteen patients did not undergo surgery. Eight of these patients were considered technically inoperable. The other seven nonsurgical patients were potentially operable but were not resected. Two of these patients refused surgery; the other five had their surgery electively deleted when no clinical evidence of disease could be found. All 15 nonsurgical patients received the planned 6000 rads of radiation.

The achievement of a complete response at the time of the first evaluation was associated with local disease extent, but not with age, histologic differentiation, or the site of tumor origin. Nine of the ten patients with $T_{1-3}N_0$ disease were complete responders at first evaluation. Five of these complete responses were pathologically and four were clinically documented. This is significantly better than the two complete responses (one clinical and one pathological) achieved in the six T_4N_0 patients ($P < 0.05$). Similarly, only six of the 19 stage IV patients achieved a complete response at first evaluation compared to ten of the 15 stage III patients ($P < 0.05$). There is no significant difference between the 11 complete responses (six pathological, five clinical) achieved in the 16 N_0 patients and the nine complete responses (four pathological, five clinical) achieved in the 22 N_{1-3} patients.

Thirty-five of the 38 patients (92%) were rendered disease-free. This includes 14 of the 15 nonsurgical patients (93%), although four of these patients have since relapsed. No patient considered free of disease after surgery has relapsed. No patients have been lost to follow-up.

The projected Kaplan-Meier 2-year disease-free survival for the entire patient cohort is 74% (fig 2). There is no statistically significant difference in disease-free survival between patients with stages I and II, stage III, and stage IV disease (P = 0.49). Fourteen of the 19

TABLE 4.—Achievement and maintenance of disease-free status*

	Evaluation 1	After surgery	Evaluation 2	Alive, disease-free†
Surgical group (N = 23)	11‡	21		21
Nonsurgical group (N = 15)	9		14	10
Inoperable (N = 8)	3		7	3
Operable (N = 7)	6		7	7

*Values = No. of patients.
†At last follow-up.
‡No pathologic evidence of disease at surgery.

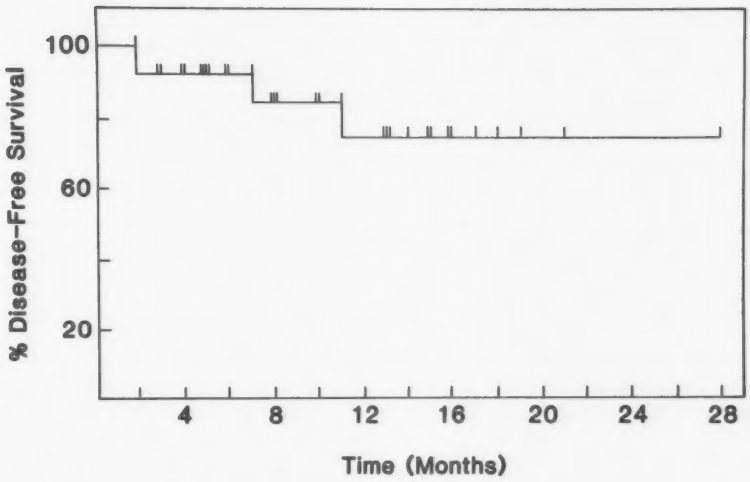


FIGURE 2.—Kaplan-Meier disease-free survival for the entire patient cohort (N = 38).

patients with stage IV disease are currently alive and disease-free. There is also no significant disease-free survival difference between the surgical and nonsurgical patient groups (P = 0.27). The median disease-free survival is > 10 months.

Treatment failures included the three patients who never achieved local control, three patients who relapsed locally, and a single patient who relapsed with metastatic disease alone. The likelihood of treatment failure was unassociated with histologic differentiation, site of origin, age, stage, tumor size (T), nodal extent (N), or the ability to deliver all of the planned therapy. It was also not significantly different between the surgical and nonsurgical groups.

Surgical complications were noted in six of the 23 surgical patients and included delayed healing in four patients and minor infection in two. A single patient developed osteoradionecrosis 1 month after completing all of his planned therapy.

DISCUSSION

The concept of neoadjuvant chemotherapy and/or radiation therapy in the treatment of squamous cell carcinoma of the head and neck has particular appeal. Initial nonoperative down-staging of a locally advanced tumor might allow for less extensive surgery as well as a better cosmetic and functional result. Whether neoadjuvant chemotherapy alone has a role remains to be determined (3,7,10). Uncontrolled trials have been promising (6,7,12-16), but the initial results of randomized studies have been unimpressive (9,17-19). One possible explanation may be the limited ability of chemotherapy to produce complete responses despite a high overall response rate (3,6,7,9-11,13,14,16). Since treatment success has been correlated with the achievement of a complete response to chemotherapy (22,23), the value of a partial response, even in a neoadjuvant setting, may be limited.

Initial radiation therapy, prior to surgical resection, has also been disappointing. Doses sufficient to produce meaningful tumor response often result in considerable treatment delay, an increased incidence of distant metastases, and an increase in surgical complications (24-26). The ability of certain chemotherapeutic agents to produce radiosensitization is, therefore, attractive. Major tumor regression might be possible with doses of radiation small enough to avoid a significant increase in surgical morbidity.

There is considerable laboratory evidence demonstrating the radiosensitizing ability of both 5-FU (27-30) and cisplatin (31-33). There is also considerable clinical data supporting a radiosensitizing role for 5-FU in several human neoplasms. Initial studies explored the use of this agent by bolus injection and were promising but limited in success. Survival benefit was nonetheless demonstrated in patients treated with bolus 5-FU and radiation in pancreatic adenocarcinoma (34) and in some head and neck cancers (35). Byfield et al (36) have more recently suggested that 5-FU may be of greater value as a radiosensitizer when given as a continuous iv infusion. A number of clinical studies have now been reported using infusional 5-FU and radiation, both with and without other chemotherapeutic agents such as mitomycin or cisplatin. High response rates have been seen in squamous cell cancer of the esophagus (37,38), head and neck (39-41), and non-small cell lung cancer (42,43). In squamous cell cancer of the anus, the response has been considered significant enough to obviate the need for radical surgery in most patients (44,45).

A clinical role for cisplatin as a radiosensitizer has been suggested by several recent trials and is under active investigation (46-49). Of more immediate interest is its demonstrated value in combination with a 5-FU infusion, without simultaneous radiation, in patients with head and neck cancer. A response rate of 93%, with 54% complete responses, has been reported in previously untreated patients (22). This chemotherapy combination in conjunction with radiation would thus seem the ideal choice for the initial treatment of this disease.

We have chosen as an endpoint the achievement of disease-free status (complete remission), as this is a clear prelude to long-term survival (13,14,16). Although patients with residual disease may have experienced palliative benefit, disease progression and death invariably followed (3,14,16). Our results are, therefore, expressed in terms of the disease-free survival rather than as survival alone. It is unclear how much meaningful survival remains for patients with head and neck cancer with residual or recurrent disease after aggressive initial therapy (3,10). Because cure seems possible in significant numbers of patients with advanced disease, it should remain the treatment goal.

Although these results are clearly preliminary, they offer new optimism for the therapy of head and neck cancer. Twenty of our 38 patients (53%) were complete responders at the time of their first evaluation. Subsequent surgery was able to render an additional ten patients disease-free, and after a second course of chemotherapy, 35 of the 38 patients (92%) were considered free of disease. Only four patients have since relapsed. The correlation of greater initial treatment success with lesser initial disease extent is expected (4,6,11,14). Surprisingly, overall disease-free survival is not significantly affected by stage. It remains a realistic probability even in those patients with stage IV disease at presentation.

The toxicity of this combined modality approach is significant. Although no treatment deaths were encountered, hospitalization was often required during chemoradiotherapy. A large percentage of patients experienced life-threatening myelosuppression, despite smaller doses of chemotherapeutic agents than those given by Decker et al (15). Presumably the radiation therapy adds to the myelosuppression as well as to the therapeutic efficacy. The mucositis encountered was often very severe, requiring hospitalization for alimentation. Radiation therapy was not interrupted because of this mucositis, however, and it resolved completely in all patients. Nonetheless, it was associated with a significant weight loss over the course of therapy. The 4-5 months required to complete treatment also posed a significant compliance barrier in this group of patients.

This success with chemotherapy and radiation therapy raises several questions concerning the role of surgery. We have found that the initial evaluation of a patient's potential operability is meaningless, given the tumor shrinkage usually achieved. Adherence to a definition of operability based on the original disease extent would have significantly reduced the number of patients proceeding to surgery. Whether this would have affected overall treatment results is unclear. However, adherence to a surgical procedure defined by the original disease extent would have been unnecessarily mutilating. Although this treatment philosophy is controversial (6,10,12, 14,16), 21 of the 23 patients who underwent surgery are currently alive and disease-free.

It is of interest that the seven initially operable patients who did not undergo surgery all remain disease-free. Six of these seven were considered disease-free at the time of their first evaluation. This raises the question of whether surgery is indeed necessary in all potentially operable patients. It may only be needed in those patients with extensive disease at presentation who do not rapidly achieve a complete response with chemoradiotherapy.

The durability of the disease-free survival achieved and the reproducibility of these results are important unanswered questions. Further studies are also necessary to determine the appropriate criteria for surgery in these patients. Although when the results are considered the morbidity of treatment is justifiable, efforts need to be directed towards reducing or ameliorating the treatment-associated toxicity.

REFERENCES

1. PROBERT JC, THOMPSON RW, and BAGSHAW MA. Patterns of spread of distant metastases in head and neck cancer. Cancer 33:127-133, 1974.
2. MERINO OR, LINDBERG RD, and FLETCHER GH. An analysis of distant metastases from squamous cell carcinoma of the upper respiratory and digestive tracts. Cancer 40:145-151, 1977.
3. HONG WK, and BROMER R. Chemotherapy in head and neck cancer. N Engl J Med 308:75-79, 1983.
4. BATAINI JP, BRUGERE J, JAULERRY CH, ET AL. Radiation treatment of lateral epilaryngeal cancer. Am J Clin Oncol 7:641-645, 1984.
5. BLUMBERG AL, FU KK, and PHILLIPS TL. Results of treatment of carcinoma of the base of the tongue, the UCSF experience, 1957-1976. Int J Radiat Oncol Biol Phys 5:1971-1976, 1979.
6. AMREIN PC, FINGERT H, and WEITZMAN SA. Cisplatin-vincristine-bleomycin therapy in squamous cell carcinoma of the head and neck. J Clin Oncol 1:421-427, 1983.
7. GLICK JH, and TAYLOR SG, IV. Integration of chemotherapy into a combined modality treatment plan for head and neck cancer: a review. Int J Radiat Oncol Biol Phys 7:229-242, 1981.
8. UCMAKLI A. The treatment of patients with stage IV squamous cell carcinoma of the head and neck. Otolaryngol Clin North Am 13:529-532, 1980.
9. TAYLOR SG, IV, APPELBAUM E, SHOWEL JL, ET AL. A randomized trial of adjuvant chemotherapy in head and neck cancer. J Clin Oncol 3:672-679, 1985.
10. MEAD GM, and JACOBS C. Changing role of chemotherapy in treatment of head and neck cancer. Am J Med 73:582-595, 1982.
11. WOLF GT, MAKUCH RW, and BAKER SR. Predictive factors for tumor response to preoperative chemotherapy in patients with head and neck squamous carcinoma. Cancer 54:2869-2877, 1984.
12. SCHULLER DE, WILSON HE, SMITH RE, ET AL. Preoperative reductive chemotherapy for locally advanced carcinoma of the oral cavity, oropharynx, and hypopharynx. Cancer 51:15-19, 1983.
13. PRICE LA, and HILL BT. Safe and effective induction chemotherapy without cisplatin for squamous cell carcinoma of the head and neck.

Med Pediatr Oncol 10:535–548, 1982.
14. PENNACCHIO JL, HONG WK, SHAPSHAY S, ET AL. Combination of *cis*-platinum and bleomycin prior to surgery and/or radiotherapy compared with radiotherapy alone for the treatment of advanced squamous cell carcinoma of the head and neck. Cancer 50:2795–2801, 1982.
15. DECKER DA, DRELICHMAN A, JACOBS J, ET AL. Adjuvant chemotherapy with *cis*-diamminedichloroplatinum II and 120-hour infusion 5-fluorouracil in stage III and IV squamous cell carcinoma of the head and neck. Cancer 51:1353–1355, 1983.
16. ADELSTEIN DJ, HINES JD, SHARAN VM, ET AL. Combination chemotherapy prior to definitive local therapy in squamous cell carcinoma of the head and neck. Oncology 42:80–85, 1985.
17. JACOBS C, WOLF GT, MAKUCH RW, ET AL. Adjuvant chemotherapy for head and neck squamous carcinomas. Proc ASCO 3:182, 1984.
18. SCHULLER DE, WILSON H, HODGSON S, ET AL. Preoperative reductive chemotherapy for stage III or IV operable epidermoid carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx, phase III, a Southwest Oncology Group study. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, Lancaster, 1984, p 48.
19. KUN LE, TOOHILL RJ, HOLOYE PY, ET AL. Effect of induction chemotherapy in advanced cancers of the upper aerodigestive tract: a prospective, randomized trial. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, Lancaster, 1984, p 43.
20. BEAHRS OH, and MYERS MH. Manual for Staging of Cancer. Philadelphia, Lippincott, 1983.
21. ROSENFELD CS, and BRODER LE. Cisplatin-induced autonomic neuropathy. Cancer Treat Rep 68:659–660, 1984.
22. ROONEY M, KISH J, JACOBS J, ET AL. Improved complete response rate and survival in advanced head and neck cancer after three-course induction therapy with 120-hour 5-FU infusion and cisplatin. Cancer 55:1123–1128, 1985.
23. ENSLEY J, KISH J, JACOBS J, ET AL. Superior survival in complete responders achieved with chemotherapy alone compared to those requiring chemotherapy and radiotherapy in patients with advanced squamous cell cancer of the head and neck. Proc ASCO 3:181, 1984.
24. CACHIN Y, and ESCHWEGE F. Combination of radiotherapy and surgery in the treatment of head and neck cancers. Cancer Treat Rev 2:177–191, 1975.
25. VANDENBROUCK C, SANCHO H, LEFUR R, ET AL. Results of a randomized clinical trial of preoperative irradiation versus postoperative in treatment of tumors of the hypopharynx. Cancer 39:1445–1449, 1977.
26. SCHULLER DE, MCGUIRT WF, KRAUSE CJ, ET AL. Symposium: adjuvant cancer therapy of head and neck tumors. Increased survival with surgery alone vs combined therapy. Laryngoscope 89:582–594, 1979.
27. HEIDELBERGER C, GRIESBACH L, MONTAG BJ, ET AL. Studies on fluorinated pyrimidines. II. Effects on transplanted tumors. Cancer Res 18:305–317, 1958.
28. VERMUND H, HODGETT J, and ANSFIELD FJ. Effects of combined roentgen irradiation and chemotherapy on transplanted tumors in mice. Am J Roentgenol 85:559–567, 1961.
29. BAGSHAW MA. Possible role of potentiators in radiation therapy. Am J Roentgenol 85:822–833, 1961.
30. VIETTI T, EGGERDING F, and VALERIOTE F. Combined effect of X-radiation and 5-fluorouracil on survival of transplanted leukemic cells. J Natl Cancer Inst 47:865–870, 1971.
31. DOUPLE EB, and RICHMOND RC. Platinum complexes as radiosensitizers of hypoxic mammalian cells. Br J Cancer 37(suppl III):98–102, 1978.
32. DOUPLE EB, and RICHMOND RC. Radiosensitization of hypoxic tumor cells by *cis*- and *trans*-dichlorodiammineplatinum(II). Int J Radiat Oncol Biol Phys 5:1369–1372, 1979.
33. SOLOWAY MS, MORRIS CR, and SUDDERTH B. Radiation therapy and *cis*-diammine-dichloroplatinum(II) in transplantable and primary murine bladder cancer. Int J Radiat Oncol Biol Phys 5:1355–1360, 1979.
34. MOERTEL CG, FRYTAK S, HAHN RG, ET AL. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil. Cancer 48:1705–1710, 1981.
35. LO TCM, WILEY AL, ANSFIELD FJ, ET AL. Combined radiation therapy and 5-fluorouracil for advanced squamous cell carcinoma of the oral cavity and oropharynx: a randomized study. Am J Roentgenol 126:229–235, 1976.
36. BYFIELD JE, CALABRO-JONES P, KLISAK I, ET AL. Pharmacologic requirements for obtaining sensitization of human tumor cells in vitro to combined 5-fluorouracil or ftorafur and X-rays. Int J Radiat Oncol Biol Phys 8:1923–1933, 1982.
37. COIA LR, ENGSTROM PF, PAUL A, ET AL. A pilot study of combined radiotherapy and chemotherapy for esophageal carcinoma. Am J Clin Oncol 7:653–659, 1984.
38. LEICHMAN L, STEIGER Z, SEYDEL HG, ET AL. Combined preoperative chemotherapy and radiation therapy for cancer of the esophagus: the Wayne State University, Southwest Oncology Group, and Radiation Therapy Oncology Group experience. Semin Oncol 11:178–185, 1984.
39. BYFIELD JE, SHARP TR, FRANKEL SS, ET AL. Phase I and II trial of five-day infused 5-fluorouracil and radiation in advanced cancer of the head and neck. J Clin Oncol 2:406–413, 1984.
40. MURTHY AK, TAYLOR SG, SHOWEL J, ET AL. Improved results with simultaneous chemotherapy and radiation in head and neck cancer. Proc ASCO 4:138, 1985.
41. KAPLAN MJ, HAHN SS, JOHNS ME, ET AL. Concomitant chemotherapy and radiation therapy in advanced head and neck cancer. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, Lancaster, 1984, p 50.
42. BYFIELD JE, STANTON W, SHARP TR, ET AL. Phase I–II study of 120-hour infused 5-FU and split-course radiation therapy in localized non-small cell lung cancer. Cancer Treat Rep 67:933–936, 1983.
43. TRYBULA M, TAYLOR SG, IV, BONOMI P, ET AL. Preoperative simultaneous cisplatin/5-fluorouracil and radiotherapy in clinical stage III, non-small cell bronchogenic carcinoma. Proc ASCO 4:182, 1985.
44. LEICHMAN L, NIGRO N, VAITKEVICIUS VK, ET AL. Cancer of the anal canal. Model for preoperative adjuvant combined modality therapy. Am J Med 78:211–215, 1985.
45. MICHAELSON RA, MAGILL GB, QUAN SHQ, ET AL. Preoperative chemotherapy and radiation therapy in the management of anal epidermoid carcinoma. Cancer 51:390–395, 1983.
46. REIMER RR, GAHBAUER R, BUKOWSKI RM, ET AL. Simultaneous treatment with cisplatin and radiation therapy for advanced solid tumors: a pilot study. Cancer Treat Rep 65:219–222, 1981.
47. AL-SARRAF M, KINZIE J, MARCIAL V, ET AL. High local and regional complete remission with the combination of *cis*-platinum and radiotherapy in unresectable head and neck cancers. RTOG study. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, Lancaster, 1984, p 50.
48. LEIPZIG B, WETMORE SJ, KLUG D, ET AL. *Cis*-platinum sensitization to radiotherapy of squamous cell carcinomas in the head and neck. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, Lancaster, 1984, p 42.
49. BLOOM EJ, GREEN MD, COOPER JS, ET AL. Concomitant use of *cis*-platinum (CDDP) chemotherapy and radiation therapy (RT) in the treatment of advanced head and neck cancer. Proc ASCO 4:137, 1985.

Intestinal Cryptosporidiosis: A Complication of Cancer Treatment[1]

Graham Michael Mead,* John William Sweetenham, David Laurence Ewins, Maria Furlong, and John Andrew Lowes[2,3]

Cryptosporidium, an intestinal protozoan parasite, is a recognized cause of sporadic, self-limiting diarrhea in normal individuals (1-3). In immunocompromised patients, infection with this organism may be associated with life-threatening gastroenteritis, particularly in patients with acquired immunodeficiency syndrome (AIDS) (2,3). We report three cases of cryptosporidiosis in patients with cancer, which indirectly resulted in the death of one patient.

CASE REPORTS

Patient 1

Patient 1 was a 67-year-old woman who presented with massive splenic and abdominal lymph node enlargement in October 1984. Biopsy revealed a malignant lymphoma of follicular, predominantly small cleaved subtype (4), and a subsequent bone marrow biopsy was found to be infiltrated with tumor. She was treated with chlorambucil (5 mg/day orally) and prednisolone (20 mg/day orally) on an intermittent schedule. Despite this treatment her disease progressed, and in December 1984 her therapy was changed to the combination chemotherapy regimen CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone). In January 1985, during an interval between chemotherapy treatments, she developed progressive diarrhea and vomiting, and on microscopy of her stool oocysts of cryptosporidium were seen. Her condition deteriorated despite iv hydration, and marked hypoalbuminemia (serum albumin, 18 g/L) was noted. Iv feeding was started and spiramycin (1 g three times per day) (5) was given for 3 weeks. Despite this, she deteriorated and died 5 weeks after admission. Cryptosporidium oocysts were found intermittently in her stool until the time of death.

Patient 2

Patient 2 was a 70-year-old woman who was found in September 1984 to have bulky abdominal and inguinal lymph node enlargement and thrombocytopenia. Biopsy of an inguinal node showed infiltration, with malignant lymphoma of diffuse large cell subtype. She was started on the CHOP chemotherapy regimen, but 5 days later developed drowsiness, followed by profuse diarrhea and vomiting. Cryptosporidium oocysts were seen in her stool. She was rehydrated iv, and her gastrointestinal symptoms were settled within 3 days. Her course was complicated by hypoalbuminemia (serum albumin, 20 g/L) and wound dehiscence. Stool cultures became negative after 10 days and she is at present well and disease-free after further chemotherapy.

Patient 3

Patient 3 was a 75-year-old woman who underwent mastectomy in June 1983 for a T3N1 breast carcinoma. She was treated postoperatively with tamoxifen, but in January 1985 was found to have lung and bone metastases. She was admitted to the radiotherapy unit and treatment with aminoglutethimide (250 mg daily) and cortisone acetate (50 mg twice a day) was started. This patient had no contact with either of the previous patients and was cared for by a different staff on another ward. During this admission a medical patient (A) (who was not immunocompromised) on her ward was found to be excreting oocysts of cryptosporidium in the stool. Enteric precautions were instituted, and an investigation into the possibility of a nosocomial source was carried out, although none was found. Patient 3 was discharged well, but then readmitted in March 1985 with a 4-week history of profuse diarrhea associated with dehydration and fever. Cryptosporidium oocysts were seen in her stool. Her serum albumin level was reduced (21 g/L). She was rehydrated and treatment with spiramycin (1 g three times per day) was started. During this second admission she was nursed in a single room; "enteric precautions" were taken. She recovered uneventfully despite being unable to tolerate spiramycin and her stool cultures became negative for crypto-

[1]Received June 20, 1985; revised Nov 15, 1985; accepted Dec 2, 1985.

[2]CRC Medical Oncology Unit (*G. M. Mead* and *J. W. Sweetenham*), Departments of Medicine I (*D. L. Ewins*) and Microbiology (*M. Furlong* and *J. A. Lowes*), Southampton General Hospital, Great Britain.

[3]We thank Professor J. M. A. Whitehouse and Dr. R. Buchanan for allowing us to report these cases.

* *Reprint requests to:* Graham Michael Mead, MD, CRC Wessex Medical Oncology Unit, Centre Block, Southampton General Hospital, Southampton SO9 4XY, Great Britain.

sporidium on two successive occasions. She remained free of diarrhea until her death from progressive cancer 3 months later. The time of onset of her diarrhea was consistent with spread from Patient A; she and Patient A had shared the same toilet facilities while they had been in the ward together.

COMMENT

Cryptosporidium is a recognized cause of diarrhea in mammals, birds, and reptiles. Routes of transmission are not fully understood. Oocysts in feces are demonstrated by specific stains and in these three patients an auramine stain was made on unconcentrated fecal smear (3). Immunosuppressed patients may develop profuse life-threatening diarrhea. Treatment is essentially supportive and patients receiving immunosuppressive drugs should have these stopped when possible. Spiramycin, a macrolide antibiotic, has been reported to produce symptomatic improvement, but excretion of the oocysts usually continues (5).

Little is known about the infective dose of the organism or the incubation period in immunocompetent humans and still less in the immunocompromised human (2). The severity of the disease seen in these patients and the possibility of person-to-person transmission suggest that enteric precautions such as those recommended by NCDC might be appropriate.

These three cases are the first reported in adults with lymphoma or solid tumors (excepting Kaposi's sarcoma). All three occurred at a time of progressive malignancy associated with recent or concurrent administration of steroids. Thus, cryptosporidium appears to be an important pathogen to consider in immunosuppressed and cancer patients with gastroenteritis.

ADDENDUM

We have seen two further cases of cryptosporidium since this article was accepted for publication. The first case occurred in a man receiving CHOP-based chemotherapy for Burkitt's lymphoma. His symptoms of diarrhea settled spontaneously when intensive treatment was interrupted for cranial prophylaxis. The second patient, with recurrent Hodgkin's disease, developed diarrhea, vomiting, and profound weight loss during intensive chemotherapy. This responded well to spiramycin, but oocyst excretion persists. There was no evidence of nosocomial spread in these cases.

REFERENCES

1. JOKIPII L, POHJOLA S, and JOKIPII AM. Cryptosporidium: a frequent finding in patients with gastrointestinal symptoms. Lancet 2:358-360, 1983.
2. CURRENT WL, REESE NC, ERNST JV, ET AL. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. N Engl J Med 308:1252-1257, 1983.
3. NAVIN TR, and JURANEK DD. Cryptosporidiosis, clinical, epidemiologic and parasitologic review. Rev Infect Dis 6:331-325, 1984.
4. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Cancer 49:2112-2135, 1982.
5. PORTNOY D, WHITESIDE ME, BUCKLEY E, ET AL. Treatment of intestinal cryptosporidiosis with spiramycin. Ann Intern Med 101:202-204, 1984.

Phase II Study of Etoposide for Carcinoma of the Prostate[1,2]

Philip J. Walther,[3,*] Stephen D. Williams,[4] Michael Troner,[5] F. Anthony Greco,[6] Robert Birch,[7] Lawrence H. Einhorn,[4] and the Southeastern Cancer Study Group

The treatment of prostate carcinoma with the epipodophyllotoxin derivative etoposide was studied in a multi-institutional phase II trial. An iv dose schedule of 130 mg/m^2 daily for 3 days every 3 weeks was utilized. A total of 36 patients were evaluable; the observed total response was 3%. Moderate toxicity (predominantly myelosuppression) was seen utilizing this treatment schedule. It is concluded that etoposide possesses little single-agent therapeutic activity for prostate carcinoma. [Cancer Treat Rep 70:771–772, 1986]

No clearly optimal chemotherapeutic regimen exists for the treatment of metastatic prostate carcinoma, and all single-agent trials thus far demonstrate only modest cytotoxic efficacy. An early phase I trial of etoposide demonstrated some activity for prostate carcinoma in anecdotal fashion (1). To determine whether etoposide possessed any substantial activity for the treatment of prostate carcinoma, a multi-institutional phase II study of etoposide was initiated by the Southeastern Cancer Study Group utilizing a parenteral dose schedule.

METHODS

Patient selection.—All patients entered in the study from 1979 to 1982 had given written informed consent. Histologic confirmation of the state of malignancy was performed. Pretreatment with other agents did not exclude patients from the study, but the patients must have recovered from any hematologic toxicity of that previous therapy. A Karnofsky performance status of > 50%, a creatinine clearance of 50 ml/minute, and a total serum bilirubin of < 2.0 mg/100 ml were minimal entry criteria. The patients must have had stage DII disease which failed hormonal manipulation, as well as either measurable disease or an elevated acid phosphatase.

Treatment regimen.—Etoposide, 130 mg/m^2 dissolved in 250 mg of normal saline, was infused over 30 minutes daily for 3 days and repeated on Day 21 of the study. If the patient had had previous radiotherapy (23 of 43 patients), the initial cycle dose was 100 mg/m^2, but was then escalated to 130 mg/m^2 if no appreciable hematologic toxicity occurred (allowable in only one patient). Dosage and schedule modification was limited to those patients whose nadir granulocyte and platelet counts dropped to < 1000/ml and 50,000/μl, respectively; the dosage was reduced 25% and delayed until acceptable counts (3500 cells/ml and 100,000/μl) were obtained. To be evaluable, all patients had to be treated for at least two cycles.

Response criteria.—Complete response was defined as the complete resolution of measurable disease. Partial response was defined as a 50% reduction of measurable disease, normalization of acid phosphatase, or 10% weight gain. Stable disease was defined as less response than stated above but no disease progression. Disease progression was defined as a 25% increase in lesion size or new lesions, or significant deterioration in symptoms, weight (10%), or Karnofsky performance status (> 20%). An interval increase of either acid phosphatase or alkaline phosphatase was insufficient criterion alone to be considered disease progression.

RESULTS

Of 43 patients entered into the study, 36 (84%) were fully evaluable at the time of final review. All patients

[1]Received Jan 30, 1985; revised Nov 12, 1985; accepted Dec 4, 1985.

[2]Supported by Public Health Service grants CA-03227, CA-28171, CA-05641, CA-20255, CA-03376, CA-23909, CA-24456, and CA-19657 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Division of Urologic Surgery, Department of Surgery, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, NC.

[4]Indiana University, Indianapolis.

[5]Miami University, FL.

[6]Vanderbilt University, Nashville, TN.

[7]Southeastern Cancer Study Group Statistical Center, Birmingham, AL.

[*]*Reprint requests to:* Philip J. Walther, MD, PhD, Division of Urologic Surgery, Department of Surgery, Duke Comprehensive Cancer Center, Duke University Medical Center, PO Box 3314, Durham, NC 27710.

TABLE 1.—Major toxic effects*

Toxic effect	Degree			
	Mild	Moderate	Severe	Life-threatening
Anemia	31	14	8	
Leukopenia	8	33	11	8
Thrombocytopenia	6	14	3	
Nausea/vomiting	11	3	3	
Genitourinary	3	—	—	
Liver	4	—	—	
CNS	—	—	3	
Skin and hair	14	6	—	
Allergy	3	—	—	

* Values = % of 40 patients evaluable for toxicity.

were appropriately entered, but four were evaluable for toxicity only. Three patients were lost to follow-up, while four had insufficient data accrued to assess response. Six patients received only one cycle for the following reasons: one had presumed allergic reaction to infusion (shortness of breath, diaphoresis, nausea), two were lost to follow-up after one cycle, two were placed on emergent radiation therapy (one for acute spinal cord compression and one for intractable bone pain), and one additional patient died after one cycle from pneumonia (not complicated by leukopenia). Of the 36 patients considered fully evaluable, no complete responses were noted, but one partial response (3%) was seen (2% of all 43 patients entered). Four patients (12%) remained stable, while disease progression was documented in 26 patients (60%). Toxicity from this dose schedule was common and moderate in intensity (predominantly myelosuppression). One patient developed life-threatening leukopenia (< 1000 cells/ml). No patients had severe thrombocytopenia (< 50,000 cells/μl). Nausea was generally well controlled. Toxic effects are summarized in table 1.

DISCUSSION

Etoposide is a semisynthetic epipodophyllotoxin with a range of activity which includes hematologic malignancies, testicular carcinoma, and small cell carcinoma of the lung (2). Early phase I experiences with etoposide have included patients with prostate carcinoma; a response was seen in one of 13 such prostate carcinoma patients (1,2). Since the inception of the study reported here, another partial response (one of five patients with an additional patient showing improvement) has been reported (3). In our study, of the 36 patients who were considered fully evaluable, only one (3%) showed any significant response, although four others (17%) achieved disease stabilization. A parenteral (as opposed to oral) route of administration, as suggested by Cavalli (4), incorporating a widely utilized intermittent repetitive dosage schedule (2) was used in this intensive treatment schedule. Toxicity was considered moderate and at the limits of severe toxicity for previously irradiated patients. Since prostate carcinoma occurs predominantly in an extremely elderly population, it seems unlikely that much improvement in response could be accrued from any further dose augmentation without unacceptable toxicity. A similar lack of therapeutic efficacy has likewise been reported utilizing a continuous-infusion regimen (5). Given the results of our substantial experience, it is concluded that neither an intensive intermittent treatment regimen nor a continuous-infusion regimen of etoposide has demonstrated any appreciable single-agent activity in the treatment of adenocarcinoma of the prostate.

REFERENCES

1. JUNGI WF, and SENN HJ. Clinical study of the new podophyllotoxin derivative, 4′-demethylepipodophyllotoxin 9-(4,6,-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213), in solid tumors in man. Cancer Chemother Rep 59:737-742, 1975.
2. SCHMOLL H. Review of etoposide single-agent activity. Cancer Treat Rev 9 (suppl A):21-30, 1982.
3. NISSEN NI, PAJAK TF, LEONE LA, ET AL. Clinical trial of VP16-213 (NSC141540) iv twice weekly in advanced neoplastic disease. Cancer 45:232-235, 1980.
4. CAVALLI F. VP-16-213 (etoposide). A critical review of its activity. Cancer Chemother Pharmacol 7:81-85, 1982.
5. TRUMP DL, and LOPRINZI CL. Phase II trial of etoposide in advanced prostate cancer. Cancer Treat Rep 68:1195-1196, 1984.

Combination Chemotherapy With Mitomycin and Vindesine in Advanced Non-Small Cell Lung Cancer: A Pilot Study by the Lung Cancer Working Party (Belgium)[1,2]

J. P. Sculier,* J. Klastersky, J. P. Dumont, G. Vandermoten, P. Rocmans, P. Libert, P. Ravez, D. Becquart, P. Mommen, and O. Dalesio[2,3]

The combination of mitomycin plus vindesine in advanced non-small cell lung cancer induced an objective response rate of 23% in 43 evaluable patients: 33% in nonpretreated patients (nine responses among 27 patients) and 6% in pretreated patients (one response among 16 patients). Toxicity was tolerable. We conclude that the regimen was a moderately active and easy to administer treatment for advanced non-small cell lung cancer. [Cancer Treat Rep 70:773–775, 1986]

Mitomycin (MMC) and vindesine (VDS) have been associated with objective response rates of 20% and 17%, respectively, when used as single agents in the treatment of non-small cell lung cancer (NSCLC) (1). Two recent studies (2,3) have reported that the combination of these two drugs resulted in objective response rates of 36% (21 responses among 59 patients) and 33% (18 responses among 55 patients). Since this combination could be an alternative to other regimens, we performed a pilot study in both nonpretreated and pretreated patients with advanced NSCLC.

PATIENTS AND METHODS

Patients with histologically proven and progressive inoperable NSCLC were eligible for this study. The following criteria were used for entry in the study: no chemotherapy or irradiation within 4 weeks prior to protocol treatment; life expectancy of at least 6 weeks; Karnofsky performance status of 60%; wbc and platelet counts of 4000 and 150,000/mm^3, respectively; serum creatinine < 2 mg/100 ml; age < 75 years; and no active neuromuscular disease. Informed consent was required.

All patients underwent a staging work-up before entering the study, including blood chemistries, chest x-rays, isotopic bone scan, liver computerized tomographic scan or echography, and evaluation of the lesion. Tumor was considered evaluable if it could be assessed without precise measurement of size and was considered measurable if clearly defined margins allowed measurement with calipers. Limited disease was defined as a tumor nonextending beyond mediastinum.

Therapy consisted of monthly courses of MMC (15 mg/m^2 iv on Day 1 in previously untreated patients, reduced to 10 mg/m^2 for those having received prior chemotherapy and/or chest irradiation) and VDS (3 mg/m^2 iv on Days 1 and 8 and on Day 15 for nonpretreated patients). After two cycles, MMC was reduced to 10 mg/m^2 and VDS was given every 2 weeks. If severe myelosuppression (wbc count < 1000/mm^3 and/or platelet count < 50,000/mm^3) was observed, the dose of MMC was reduced by 20% for the next courses. The dose of VDS was reduced by 25% if moderate myalgias or paresthesias occurred and by 50% if it was severe. On Days 8 and 15 of the cycles, a full dose of VDS was given if wbc and platelet counts were > 3000 and 75,000/mm^3, respectively; the dose was reduced by 50% if the counts were between 2000 and 3000 and/or 50,000 and 75,000/mm^3, respectively. VDS was not administered if the counts were < 2000 and/or 50,000/mm^3, respectively. Treatment was discontinued

[1]Received Aug 26, 1985; revised Dec 10, 1985; accepted Dec 13, 1985.

[2]This study was conducted by the Lung Cancer Working Party (Belgium) (*J. Klastersky*, chairman; *J. P. Sculier*, secretary; *P. Mommen*, data manager; and *O. Dalesio*, statistician). The following members participated in the study: Institut Jules Bordet, Bruxelles, Belgium (*J. Klastersky, J. P. Sculier, J. P. Dumont, Y. Kenis, J. J. Body, P. Van Houtte, T. H. Nguyen*, and *P. Mommen*); A. Z. Middelheim, Antwerpen, Belgium (*D. Becquart*); A. Z. Antwerpen, Belgium (*D. Becquart* and *P. Yermeire*); Hôpital de Warquignies, Boussu, Belgium (*P. Libert* and *P. Ravez*); Hôpital Brugmann, Bruxelles, Belgium (*J. Devriendt* and *J. Schmerber*); and Hôpital Erasme, Bruxelles, Belgium (*P. Rocmans, G. Vandermoten, A. Verhest, P. Ketelband*, and *J. C. Yernault*).

[3]We thank Dr. Bayssas, the Lilly Company, and Bristol Benelux for their support.

*Reprint requests to: J. P. Sculier, MD, Institut Jules Bordet, 1 rue Heger-Bordet, 1000 Bruxelles, Belgium.

TABLE 1.—Patient characteristics and response*

	Non-pretreated	Pretreated
No. of patients	31	16
Sex (M/F)	26/5	13/3
Mean age in yrs (range)	61 (40-75)	59 (46-69)
Mean performance status (range)	75% (60%-100%)	71% (60%-100%)
Loss of body weight (range)	2.8% (0%-20%)	3.6% (0%-15%)
Histology		
Squamous cell carcinoma	17	10
Adenocarcinoma	12	2
Large cell carcinoma	1	3
Other	1	1
Type of lesions		
Evaluable/measurable	17/14	6/10
Limited/disseminated	9/22	5/11
Response		
Inevaluable	3	—
Early death	1	—
Evaluable for response	27	16
Complete response	1	—
Partial response	8	1
No change	5	5
Disease progression	13	10

*Unless otherwise specified, values = No. of patients.

if there was no recovery from the toxic effects described, if leukopenia and/or thrombocytopenia persisted despite dose reduction, or if severe neurotoxicity occurred.

Patients were evaluated after two courses of therapy by a staging procedure similar to that performed prior to treatment. Complete and partial remission were defined using standard criteria. All responses were evaluated during regular meetings of the Group and by at least three physicians who did not belong to the institution of the investigator. Patients with response and no change were continued on therapy until disease progression or unmanageable toxicity. Those patients with progressive disease were considered failures. Duration of response and survival was calculated from the first day of treatment. Survival curves were calculated by the method of Kaplan and Meier. The log-rank method was used to test the statistical significance of the difference between survival curves.

RESULTS

Among the 47 registered patients (all evaluable for survival), four were inevaluable for response for the following reasons: protocol violation, early death, treatment discontinued after one course because of neurotoxicity, and second-course refusal. All of these patients had not received prior chemotherapy.

Table 1 shows characteristics of the entered patients. Sixteen patients were pretreated; four had received prior chest irradiation (usually 40-50 Gy on the primary lesion and the mediastinum); nine had received chemotherapy (usually a combination of cisplatin and etoposide); and three had received both. Thirty-three patients had disseminated disease and 14 had limited disease.

Ten objective responses were observed among 43 evaluable patients (23%; confidence limits, 11%-36%). Nine responses were observed in nonpretreated patients (33%; confidence limits, 16%-51%), including the complete response. Only one patient among those with prior therapy presented with partial response (6%; confidence limits, 0%-18%). A median number of three courses has been given (range, one to nine).

Median survival time (MST) was 21 weeks, with < 20% survival at 1 year. Survival of nonpretreated patients (MST, 22 weeks) is not statistically significant from that of pretreated patients (MST, 19 weeks), despite a better appearance of the curve ($P = 0.13$). Median duration of response was 24 weeks and median survival of responders was 40 weeks.

Toxicity consisted mainly of myelosuppression and peripheral neuropathy (table 2). Nausea and vomiting and alopecia were mild. No toxic death was observed.

TABLE 2.—Toxicity*

	Grade†				
	0	I	II	III	IV
			Nonpretreated patients		
Wbc count depression	9	3	10	5	1
Platelet count depression	26	1	—	1	—
Nausea and vomiting	20	8	3	—	—
Neurological	23	5	—	2	1
			Pretreated patients		
Wbc count depression	8	4	4	—	—
Platelet count depression	16	—	—	—	—
Nausea and vomiting	11	4	1	—	—
Neurological	10	3	2	1	—

*Values = No. of patients.
†World Health Organization toxicity scale.

There was no major difference between pretreated and nonpretreated patients.

DISCUSSION

Our results with MMC and VDS used as a first-line therapy confirmed the data previously reported (2-4). The results are in the range of the best response rates published for advanced NSCLC: 30%-40% objective response rates, with about 5% complete remissions (5). However, when given as second-line therapy, after chest irradiation and/or another combination chemotherapy (cisplatin and etoposide), we obtained very poor results. This observation suggests that MMC and VDS should only be used in nonpretreated patients with advanced NSCLC.

MMC has been studied in combination with other vinca alkaloids such as vinblastine, and relatively good results have been reported when cisplatin was added to the association: 24%-54%. A way to improve the effectiveness of the regimen would be the addition of another drug. A 21% objective response rate has been reported with 5-FU and response rates of 52% and 38% have been reported in two studies conducted with cisplatin. Further randomized studies will be necessary to compare the effectiveness of this new regimen to other chemotherapy combinations in advanced NSCLC.

REFERENCES

1. BAKOWSKI MT, and CROUCH JC. Chemotherapy of non-small cell lung cancer: a reappraisal and a look to the future. Cancer Treat Rev 10:159-172, 1983.
2. LUEDKE D, LUEDKE S, MARTELLO O, ET AL. Response of non-small cell lung cancer (NSCLC) to vindesine (VDS) and mitomycin C (mito): a Southeastern Cancer Study Group pilot study. Proc ASCO 19:190, 1983.
3. KRIS MG, GRALLA RJ, KELSEN DP, ET AL. Trials adding mitomycin to vindesine and to vindesine plus cisplatin in non-small cell lung cancer. Proc ASCO 20:225, 1984.
4. KRIS MG, GRALLA RJ, KELSEN DP, ET AL. Trial of vindesine plus mitomycin in stage-3 non-small cell lung cancer. An active regimen for outpatient treatment. Chest 87:368-372, 1985.
5. SCULIER JP, and KLASTERSKY J. Progress in chemotherapy of non-small cell lung cancer. Eur J Cancer Clin Oncol 20:1329-1333, 1984.

High-Dose Oral Medroxyprogesterone Acetate in Heavily Pretreated Patients With Metastatic Breast Cancer[1]

Paul E. Goss,[2,3,4] Susan Ashley,[2] Trevor J. Powles,[2] and R. Charles Coombes[2,4,5,*]

Fifty heavily pretreated postmenopausal patients with advanced breast cancer were treated with oral medroxyprogesterone acetate (1 g) daily for 1–18 months. Eight patients (16%) responded to treatment for periods up to 18 months and in an additional eight patients (16%) the disease stabilized for up to 10 months. Thirty-two patients (64%) developed adverse effects and eight (16%), all of whom had extensive pulmonary metastases, died a respiratory-related death thought possibly to have been brought on or accelerated by treatment. Only eight patients (16%) experienced increased appetite, weight gain, feeling of well-being, or improved performance status. We conclude that medroxyprogesterone acetate is useful in patients with advanced metastatic disease but should be restricted to carefully selected patients and used only with extreme caution in patients with underlying cardiovascular or respiratory disease. Its major application probably lies in the benefit it confers earlier in the disease. [Cancer Treat Rep 70:777–779, 1986]

Medroxyprogesterone acetate (MPA) has been shown to be an effective treatment in the management of breast cancer when given in high doses. Overall objective responses have been obtained in 28%–66% of patients receiving parenteral therapy depending on their clinical characteristics (1). Heavily pretreated patients (1–3) and patients resistant to endocrine therapy or chemotherapy both seem to respond to therapy (4). Most investigators have reported a significant increase in performance status, increase in appetite and weight, and a general sense of well-being (3).

MPA has therefore been recommended not only as a first- or second-line treatment but also as an effective and safe therapy with beneficial systemic effects in heavily pretreated and treatment-resistant disease. Our aim was to investigate whether the benefits of high-dose oral MPA outweighed the disadvantages in treatment of advanced metastatic disease.

PATIENTS AND METHODS

Fifty postmenopausal women (37–82 years of age) with histologically proven metastatic breast cancer were treated with oral MPA (1 g/day for ⩽ 18 months). Standard International Union Against Cancer criteria were used to assess disease response (5). All patients were fully staged prior to and at 2–3 and 6–8 months after the start of treatment (6). All patients were heavily pretreated, the mean number of treatments being 2.6/patient (range, one to six treatments). Of 43 evaluable patients, 42 (98%) had received at least one previous course of endocrine therapy (mean, 1.8/patient), of whom 12 had responded and 33 had not. Nineteen patients (45%) had previously received chemotherapy (mean, 0.8/patient). Median time from first relapse to start of MPA was 27 months. Estrogen receptor (ER) content of the primary tumor was measured by the method of McGuire et al (7) in 22 of the 43 evaluable patients. ER-positive tumors were designated those which contained > 15 fmols of estradiol/mg of cytosol protein. Patients selected had advanced disease, with an average of 2.5 sites of disease. Pretreatment sites of metastases were: soft tissue (34), lung parenchyma (18), liver (11), bone (32), and pleural effusions (nine). Toxicity was evaluated by means of a standard questionnaire completed monthly.

[1]Received May 17, 1985; revised Dec 16, 1985; accepted Dec 26, 1985.

[2]Cancer Research Campaign Laboratory, Institute of Cancer Research (*P. E. Goss*), Royal Marsden Hospital (*P. E. Goss, S. Ashley, T. J. Powles*, and *R. C. Coombes*), Sutton, Surrey, England.

[3]P. E. Goss is the recipient of a CRC Fellowship.

[4]Ludwig Institute for Cancer Research (London Branch), St. George's Hospital Medical School, England.

[5]We thank Sarah Clarke and Jackie Gibb for research assistance.

*Reprint requests to: Dr. R. Charles Coombes, Ludwig Institute for Cancer Research (London Branch), St. George's Hospital Medical School, Blackshaw Rd, London SW17 0QT, England.

TABLE 1.—Characteristics of evaluable patients treated with MPA*,†

Characteristic	Responders	Stable disease	Progressive disease
Evaluable (%)	8 (19)	8 (19)	27 (63)
Mean age in yrs (range)	69 (64-75)	65 (51-77)	60 (37-78)
ER status			
Positive/negative/unknown	4/0/4	2/3/3	10/3/14
Previous therapy			
Endocrine			
Total	8	8	26
Responders	4	1	7
Chemotherapy			
Total	2	3	14
Responders	0	2	6

*1 g/day orally.
†Unless otherwise specified, values = No. of patients.

RESULTS

Seven of the 50 patients treated were unevaluable because they died within 3 weeks after the initiation of treatment prior to being adequately restaged. Sixteen percent of all patients receiving therapy responded. Eight (19%) of the 43 evaluable patients responded. A breakdown of mean age, ER status, and response to previous endocrine and chemotherapy is given for patients responding, having stable disease, or having disease progression in table 1.

Concerning sites of response, soft tissue sites responded in seven of 30 (26%) patients and only a minority of other sites responded. None of the pulmonary metastases responded to therapy. There was no difference in the time from first relapse to the start of MPA between responders versus nonresponders.

The major adverse effects which occurred during treatment were increasing breathlessness (12 of 43 patients; 24%) and associated edema (12 of 43 patients; 24%). Reduction in dosage in ten patients and cessation in six, together with diuretic therapy, led to a symptomatic improvement. Nausea (four patients), vomiting (two), depression (four), and alopecia (two) were less common side effects of treatment. Only eight (16%) patients experienced improved appetite, feeling of well-being, or improved performance status (Karnofsky).

DISCUSSION

All patients but one in this study had been previously treated with various types of systemic therapy. Therefore, the relatively low objective response rate probably relates to the extent of previous treatment. However, only eight patients experienced palliative benefits from treatment, whereas 12 developed increasing breathlessness on treatment. Reductions in dosage (6) and treatment with diuretics (8) alleviated these symptoms in ten patients, but an additional two patients failed to respond and died. Furthermore, of the seven patients who were not evaluable, six had parenchymatous pulmonary metastases with associated carcinomatous lymphangitis and died at home from respiratory failure within 2 weeks of starting treatment. These deaths cannot be directly attributed to therapy but rather may have been due to progressive disease. Nevertheless, in view of the absence of response of pulmonary metastases and the possibility of significant fluid retention, MPA therapy is probably best avoided in patients with pulmonary metastases, in particular lymphangitis, and in patients with underlying cardiovascular or benign respiratory disease. Response according to ER status cannot be adequately assessed from this study in view of the large number of missing values and the fact that ER values obtained were from primary tumors prior to a number of subsequent endocrine therapies.

We conclude that in heavily pretreated patients, MPA produces relatively low response rates without the palliative benefits reported for earlier treatment. Its major application probably lies in the response rates and benefits it confers earlier in the disease.

REFERENCES

1. MATTSON W. Current status of high dose progestin treatment in advanced breast cancer. Breast Cancer Res Treat 3:231, 1983.
2. LOBER J, ROSE C, SALIMTSCHIK M, ET AL. Treatment of advanced breast cancer with progestins. A review. Acta Obstet Gynecol Scand (suppl) 101:39, 1981.
3. GANZINA F. High dose medroxyprogesterone acetate (MPA) treatment in advanced breast cancer. A review. Tumori 65:563, 1979.
4. DE LENA M, BRAMBILLA C, VALAGUSSA P, ET AL. High-dose medroxyprogesterone acetate in breast cancer resistant to endocrine and cytotoxic therapy. Cancer Chemother Pharmacol 2:175, 1979.
5. HAYWARD JL, CARBONE PP, HEUSON J-C, ET AL. Assessment of response to therapy in advanced breast cancer. Cancer 39:1289, 1977.
6. COOMBES RC, DEARNALEY D, HUMPHREYS J, ET AL. Danazol treatment of advanced breast cancer. Cancer Treat Rep 64:1073-1076, 1980.

7. MCGUIRE WL, CARBONE PP, and SEARS ME. Estrogen receptors in human breast cancer. New York, Raven Press, 1975, pp 1–7.
8. JONAT W, KNAPP W, SCHUMANN B, ET AL. High-dosed oral gestagen therapy as a non-responder regimen in metastatic breast cancer. Dtsch Med Wochenschr 109:46, 1984.

Phase II Trials of 5-FU, Doxorubicin, and Cisplatin in Advanced, Measurable Adenocarcinoma of the Lung and Stomach[1,2]

Eduardo L. Cazap,[3] Christian Gisselbrecht,[4] Frederick P. Smith,[5,6] Roberto A. Estevez,[3] Carlos A. Alvarez,[7] Claude Lagarde,[8] Adrian Hannois,[7] Susan Ahmed,[5] Philip S. Schein,[5,9] and Paul V. Woolley[5,*]

A combination of 5-FU (600 mg/m² on Days 1 and 8), doxorubicin (40 mg/m² on Day 1), and cisplatin (75 mg/m² on Day 1) has been used for treatment of 31 patients with advanced measurable adenocarcinoma of the lung and 35 with gastric cancer. The regimen was given every 4 weeks until disease progression to patients who had not received prior chemotherapy. One complete response occurred in the lung cancer group. Ten of the gastric cancer patients (29%) had partial responses. The median duration of response was 5.5 months and the median survival in responding patients was 10.8 months. Toxicity of the regimen was moderate. We conclude that this combination offers no particular advantages over previously described treatments for these diseases. [Cancer Treat Rep 70:781–783, 1986]

This trial examined the use of cisplatin in combination with 5-FU and doxorubicin (FAP) for previously untreated, advanced adenocarcinoma of the stomach and lung. The rationale of the study was based upon reports of cisplatin activity in lung cancer, particularly in untreated patients (1–4), and also on early indications from the European Organization for Research on Treatment of Cancer of single-agent activity in gastric cancer.

PATIENTS AND METHODS

Between March 1979 and March 1981, 35 patients with advanced gastric cancer and 31 with lung cancer were entered into this protocol. All patients had biopsy-proven disease that was measurable by palpation, roentgenogram, radionuclide scan, or computerized axial tomographic scan. None had been treated previously with chemotherapy. The FAP regimen was administered on the following schedule: 5-FU, 600 mg/m² on Days 1 and 8; doxorubicin, 40 mg/m² on Day 1; and cisplatin, 75 mg/m² on Day 1. The regimen was repeated at 4-week intervals. Cisplatin was withheld for a creatinine clearance < 60 ml/min and doxorubicin was withheld for bilirubin > 3.0 mg/dl.

RESULTS

One of the lung cancer patients treated with FAP had a complete response lasting 5.5 months, but no partial remissions were seen (table 1). Nine patients (29%) had minor responses and six (19%) had stable disease for at least 3 months while on treatment. These patients are grouped together under "stable disease" in table 1. The median survival of the lung cancer patients was 10 months.

Ten (29%) of the 35 gastric cancer patients achieved partial remission, three had minor responses, and five had stabilization of disease (table 1). None had a complete remission. The median time to progression was 7 months (range, 3–10), while the median duration of survival in all treated patients was 6 months (range, 1–18).

The combination was generally well tolerated. Most patients had some nausea and vomiting on the first day of therapy but it was less than originally anticipated. There

[1]Received Mar 11, 1985; revised Dec 19, 1985; accepted Jan 6, 1986.

[2]This study was a collaborative effort of the Vincent T. Lombardi Cancer Research Center, the Catedra de Oncologia de la Universidad del Salvador (Buenos Aires), and the Fondation Bergonie (Paris) under the auspices of the National Cancer Institute-Pan American Health Organization Collaborative Cancer Research Treatment Program (contract N01-CO-65332 from the Office of the Director, National Cancer Institute, National Institutes of Health, Department of Health and Human Services); and of the French-American Cancer Research Agreement.

[3]Catedra de Oncologia, Universidad del Salvador, Buenos Aires, Argentina.

[4]Hôpital Saint-Louis, Paris, France.

[5]Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC.

[6]*Present address:* The Washington Clinic, Washington, DC.

[7]Universidad del Salvador, Central Military Hospital, Buenos Aires, Argentina.

[8]Fondation Bergonie, Bordeaux, France.

[9]Smith, Kline and French, Philadelphia, Pa.

[*]*Reprint requests to:* Paul V. Woolley, MD, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 1.—Characteristics of patients treated with FAP protocol*

Characteristic	Lung cancer patients	Gastric cancer patients
Sex (M/F)	25/6	25/10
Median age (yrs)	59	57
Performance status (ECOG)†		
0	12	7
1	12	13
2	4	9
3	1	3
4	1	3
Not recorded	3	—
Site of measurable disease‡		
Chest x-ray	28	1
Bone scan	5	—
Liver scan	1	15
Distant modes	2	6
Abdominal mass	—	13
Response		
Complete	1	0
Partial	0	10
Stable disease	15	8
Progressive disease	15	17
Median time to progression of responder(s) (mos)	5.5	7 (range, 3-10)
Median survival of all patients treated (mos)	10	6 (range, 1-18)

* Unless otherwise specified, values = No. of patients.
† ECOG = Eastern Cooperative Oncology Group.
‡ Some patients had > 1 site of measurable disease.

were two patients with severe mucositis, one with a febrile episode, one with sepsis, and one with hearing loss. Seventeen patients developed total alopecia. Leukocyte counts < 1500/mm^3 occurred in three of the 66 patients, and platelet counts < 30,000/mm^3 occurred in one. The median nadir leukocyte count was 3800/mm^3 (range, 1100-11,000) and the median nadir platelet count was 150,000/mm^3 (range, 27,000-420,000). One patient with a wbc count of 1800/mm^3 and a platelet count of 27,000/mm^3 required hospitalization due to gastrointestinal and nasal bleeding. No cardiac or chronic nephrotoxicity was encountered.

DISCUSSION

The activity of 5-FU, doxorubicin, and mitomycin (FAM) in gastric and lung adenocarcinoma has been previously demonstrated (5,6). Since the original description of this regimen in gastric cancer, several attempts have been made to modify it to produce better quality responses and greater long-term survival (7,8). In the present phase II study, the absolute response rate in gastric cancer was lower than previously reported for FAM (5), and also the duration of these responses was short. The median survival of the gastric cancer group was only 6 months. While statistical comparisons of FAP with other regimens should be addressed through the mechanism of a phase III trial, there is no evidence from the current study that FAP has improved the overall response rates or survival of treated patients as compared to FAM. At the same time, the incorporation of cisplatin into this regimen has converted FAM from a readily manageable outpatient combination to one that requires more hydration and has less ease of handling. It is possible, however, that another combination could be studied employing 5-FU, doxorubicin, mitomycin, and cisplatin, since the toxicity of FAP has been less than expected. Nausea and vomiting were well controlled and the myelosuppression of the regimen has been acceptable. It is of interest that another report of a FAP regimen for gastric cancer has reported severe hematologic toxicity using similar drug doses (9). We cannot explain the disparity between the results of the two groups. In the present study cisplatin was given on an inpatient schedule that included 24 hours of hydration, while the Eastern Cooperative Oncology Group study used an outpatient cisplatin regimen. Whether this accounts for the differences is not clear, but other groups have reported acceptable toxicity with similar combinations (10,11).

In summary, while the FAP regimen has some activity in gastric cancer, it failed to demonstrate any significant activity in lung cancer. The identification of new agents with cytotoxic activity remains critical to therapeutic advances in these diseases.

REFERENCES

1. EAGAN RT, INGLE JN, FRYTAK S, ET AL. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat Rep 61:1339-1345, 1977.
2. HIGBY DJ, WILBUR D, WALLACE HJ, JR. ET AL. Adriamycin-cyclophosphamide and adriamycin-*cis*-dichlorodiammineplatinum(II) combination chemotherapy in patients with advanced cancer. Cancer Treat Rep 61:869-873, 1977.
3. VOGL S, OHNUMA T, PERLOFF M, ET AL. Combination chemotherapy with adriamycin and *cis*-dichlorodiammineplatinum(II) in patients with neoplastic diseases. Cancer 38:21-26, 1976.
4. BRITELL JC, EAGAN RT, INGLE JN, ET AL. *cis*-Diamminedichloroplatinum(II) alone followed by adriamycin plus cyclophosphamide at progression versus *cis*-dichlorodiammineplatinum(II), adriamycin and cyclophosphamide in combination for adenocarcinoma of the lung. Cancer Treat Rep 62:1207-1210, 1978.
5. MACDONALD JS, SCHEIN PS, WOOLLEY PV, ET AL. 5-Fluorouracil, doxorubicin and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. Ann Intern Med 93:533-536, 1980.
6. BUTLER TP, MACDONALD JS, SMITH FP, ET AL. 5-Fluorouracil, adriamycin and mitomycin-C (FAM) chemotherapy for adenocarcinoma of the lung. Cancer 43:1183-1188, 1979.
7. WOOLLEY PV, MACDONALD JS, SMYTHE T, ET AL. A Phase II trial of ftorafur, adriamycin and mitomycin-C (FAM II) in advanced gastric adenocarcinoma. Cancer 44:1211-1214, 1979.
8. GISSELBRECHT C, SMITH FP, MACDONALD JS, ET AL. The effect of sequential addition of the nitrosourea, chlorozotocin, to the FAM combination in advanced gastric cancer. Cancer 51:1792-1794, 1983.
9. VOGL SE, and ENGSTROM PF. Cisplatin, doxorubicin, and 5-FU in

combination for advanced gastric cancer. Cancer Treat Rep 68:1273-1275, 1984.
10. WILLIAMS SD, DONOHUE JP, and EINHORN LH. Advanced bladder cancer: therapy with *cis*-dichlorodiammineplatinum(II), adriamycin, and 5-fluorouracil. Cancer Treat Rep 63:1573-1576, 1979.
11. RUCKDESCHEL JC, MEHTA CR, SALAZAR OM, ET AL. Chemotherapy for inoperable non-small cell bronchogenic carcinoma: EST 2575, generation II. Cancer Treat Rep 65:965-972, 1981.

Modified Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, and Cytarabine (COMLA) in Intermediate- and High-Grade Lymphoma: An Effective Short-Course Regimen[1,2]

Maria R. Baer,* Richard S. Stein, John P. Greer, Steven N. Wolff, John D. Hainsworth, and John M. Flexner[3]

Twenty-one patients with intermediate- and high-grade lymphomas were treated with modified cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine (COMLA). This regimen utilizes the drugs used in COMLA in similar doses, but cyclophosphamide is given twice as frequently, cytarabine is given by continuous infusion, and the total duration of therapy is reduced to 16 weeks. Complete remission rates were 80% in patients with intermediate-grade lymphomas and 67% in patients with high-grade lymphomas. Persistent complete remission rates were 60% and 33%, respectively, with a median follow-up of 26 months for surviving patients. This regimen was myelotoxic, but there were no toxic deaths. Modified COMLA is a relatively nontoxic regimen producing excellent results in intermediate-grade lymphomas. [Cancer Treat Rep 70:785–787, 1986]

The regimen of cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine (COMLA) is moderately effective in the treatment of diffuse histiocytic lymphoma (DHL) (1). This study employed a pharmacologic modification of the COMLA regimen in which the interval between cyclophosphamide doses was decreased from 13 to 6 weeks and cytarabine was administered as a 5-day continuous infusion rather than as eight weekly bolus injections. These changes resulted in a shorter duration of therapy, 16 weeks compared to 37 weeks for COMLA. Because of our concern that this regimen might be undertreatment, we attempted to exclude patients belonging to unfavorable pathologic subsets (1) or having unfavorable clinical features, specifically bone marrow involvement or masses > 10 cm (2).

PATIENTS AND METHODS

Twenty-one previously untreated patients received "modified COMLA" between March 1981 and April 1984. The patients with high-grade lymphomas and six patients with intermediate-grade lymphoma and unfavorable clinical features (large abdominal masses and/or bone marrow involvement) entered the study only after their primary physicians elected not to treat them with more toxic regimens. Patients underwent clinical staging, including abdominal computerized tomographic scan and bone marrow aspiration and biopsy. Assignment of stage IV required histologic proof. Complete remission required normalization of all abnormal baseline studies.

Pathologic material was routinely studied both as tissue sections and as cell suspensions as previously reported (3). Three patients whose diagnosis was established before referral to Vanderbilt University Hospital and two others whose pathologic material did not allow for classification as to immunologic origin were included in the study as large transformed cell lymphoma of indeterminate origin.

The modified COMLA regimen consisted of cyclophosphamide (1500 mg/m^2 iv on Day 1), vincristine (1.4 mg/m^2 on Days 1, 8, and 15), cytarabine (100 mg/m^2/day for 5 days by continuous infusion starting on Day 22), and methotrexate (120 mg/m^2 iv on Day 27), with leucovorin (25 mg/m^2 orally every 6 hours for four doses) starting 24 hours after the dose of methotrexate. Cycles were repeated every 43 days for three cycles. Administration of cyclophosphamide and/or cytarabine was delayed if the wbc count was < 3000/mm^3 or the platelet count was < 100,000/mm^3. Patients were hospitalized overnight for administration of cyclophosphamide and

[1]Received Oct 30, 1985; revised Dec 31, 1985; accepted Jan 15, 1986.

[2]Supported in part by Public Health Service grant AM-07186 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services.

[3]Divisions of Hematology (*M. R. Baer, R. S. Stein, J. P. Greer,* and *J. M. Flexner)* and Oncology (*S. N. Wolff* and *J. D. Hainsworth*), Department of Medicine, Vanderbilt University Hospital and Veterans Administration Medical Center, Nashville, TN.

* *Reprint requests to:* Dr. Maria R. Baer, Division of Hematology, Department of Medicine, Vanderbilt University Medical Center, Rm A-3311 MCN, Nashville, TN 37232.

again for the 5-day infusion of cytarabine.

Eight patients received radiotherapy to local tumor masses (table 1). Two patients received two and three cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), respectively, as consolidation at the discretion of their primary physician. Although this was a protocol violation, we did not exclude these patients from data analysis.

Life table analysis (4) is based on status as of July 1985.

RESULTS

Complete response (CR) was achieved in 12 of 15 patients (80%) with intermediate-grade lymphoma, two of four (50%) with small transformed cell lymphoma, and two of two with other high-grade lymphomas (table 1). Nine of 12 patients (75%) with intermediate-grade lymphomas achieving CR remain in CR (median follow-up, 26 months). We project a 60% 2-year *relapse-free survival* for these patients. The 2-year actuarial *survival* for patients with intermediate-grade lymphoma is 66%. Of the two patients with small transformed cell lymphoma achieving CR, one relapsed and the other died of acute leukemia. Both patients with other high-grade lymphomas remain in CR.

All patients developed neutropenia (granulocyte count $< 1000/mm^3$), and eight patients had 11 episodes of fever requiring hospitalization, including two episodes of gram-negative sepsis. Seven patients required rbc transfusions and two required platelet transfusions. Four patients experienced sensory neuropathy and one developed Raynaud's phenomenon. One patient had a transient rise in serum creatinine to a peak of 3.7 mg/dl following the first dose of methotrexate. There were no toxic deaths. Patient 21 (table 1) developed a myelodysplastic syndrome 1 month following completion of all chemotherapy and died of acute myelomonocytic leukemia (confirmed by histochemical studies) 6 months later.

Eleven of 21 patients finished therapy within 2 weeks of the projected time interval. Only three patients required delays of > 4 weeks due to cytopenias.

DISCUSSION

Although follow-up is limited and our patient population was partially selected to include favorable cases, our results in intermediate-grade lymphoma are similar to

TABLE 1.—Patient characteristics and response to modified COMLA

Patient No.	Age (yrs)/ sex	Stage	Response*	Duration (mos)	Survival (mos)	Additional therapy
			INTERMEDIATE-GRADE LYMPHOMA			
			Large transformed cell lymphoma—B-cell type			
1	62/M	IIEA	CR	34+	34+	4000 rads to thigh†
2	50/F	IIEB	CR	23+	23+	5000 rads to forearm†
3	48/F	IIA	CR	31+	31+	
4	41/M	IIA	CR	20+	20+	
5	47/F	IIIA	CR	30+	30+	
6	71/M	IIIA	CR	28+	28+	
7	70/F	IVA	PR	2	2	
8	56/M	IVB	CR	6	20+	
9	58/M	IVB	NR	—	3	
10	61/M	IVA	CR	2	17	4000 rads to skin of lower legs
			Large transformed cell lymphoma—indeterminate			
11	74/F	IVB	CR	12+	12+	4400 rads to L4
12	68/M	IVB	CR	6	6	
13	62/M	IIIA	CR	26+	26+	
14	44/F	IIB	CR	20+	20+	3000 rads to Waldeyer's ring
15	58/F	IVB	NR	—	3	5580 rads to axilla and neck
			HIGH-GRADE LYMPHOMA			
			Peripheral T-cell lymphoma			
16	51/M	IVB	CR	24+	24+	4000 rads to sinuses, CHOP × 3
			Immunoblastic sarcoma of B cells			
17	48/M	IIIB	CR	30+	30+	
			Small transformed cell lymphoma			
18	34/M	IVB	PR	3	5	1200 rads to pharynx
19	64/M	IIIB	PR	—	3	
20	34/F	IVB	CR	13	47+	
21	60/M	IVA	CR	10+	10	Surgical resection of all known disease, CHOP × 2

*PR = partial response; NR = no response.
†Received only 2 cycles of chemotherapy.

those achieved with regimens such as M-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone), ProMACE/MOPP (prednisone, methotrexate, doxorubicin, cyclophosphamide, and etoposide/mechlorethamine, vincristine, procarbazine, and prednisone), and COP-BLAM (cyclophosphamide, vincristine, prednisone, bleomycin, doxorubicin, and procarbazine). However, the modified COMLA regimen was less toxic than those regimens. Myelotoxicity was significant, but all septic episodes were managed successfully and there were no toxic deaths. The major inconvenience of the modified COMLA regimen is the need for hospitalization to administer the 5-day infusion of cytarabine.

Recently, favorable results have been reported with MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) (5), which is also administered over a short period of time. Our study, in conjunction with the MACOP-B study, supports the hypothesis that relatively short courses of effective therapy can produce cures in DHL as in other malignancies.

We believe that modified COMLA is a rational modification of the original COMLA regimen and that it could be employed in patients in whom an anthracycline is contraindicated or as part of alternating therapy with an anthracycline-containing combination. Based on our case selection, we cannot recommend use of the regimen except in the intermediate-grade subset of DHL. While there has been a trend to study more toxic therapeutic regimens, such an approach may not be needed for all patients with DHL. Indeed, further studies to establish biologically homogeneous subsets of DHL may provide for a rational stratification of patients, not only with respect to prognostic features but also with respect to choice of therapy.

REFERENCES

1. SWEET DL, COLLINS RD, STEIN RS, ET AL. Prognostic significance of the Lukes and Collins classification in patients treated with COMLA. Cancer Treat Rep 66:1107-1111, 1982.
2. FISHER RI, DEVITA VT, JR, JOHNSON BL, ET AL. Prognostic factors for advanced diffuse histiocytic lymphoma following treatment with combination chemotherapy. Am J Med 63:177-182, 1977.
3. WRIGHT EP, GLICK AD, YORK JC, ET AL. Unclassified hematopoietic and lymphoid neoplasms: clinicopathologic features. *In* Tumors of Unknown Origin and Poorly Differentiated Neoplasms (Fer M, et al, eds). Orlando, FL, Grune and Stratton, 1985, in press.
4. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
5. KLIMO P, and CONNORS JM. MACOP-B chemotherapy for the treatment of diffuse large cell lymphoma. Ann Intern Med 102:596-602, 1985.

Use of Cisplatin, Doxorubicin, and Cyclophosphamide To Treat Advanced and Recurrent Adenocarcinoma of the Endometrium[1]

Kenneth C. Hancock,[2,3] Ralph S. Freedman,[2,*] Creighton L. Edwards,[2] and Felix N. Rutledge[2]

Eighteen patients with recurrent or advanced adenocarcinoma of the endometrium were treated with the combination of cisplatin, doxorubicin, and cyclophosphamide between January 1981 and December 1983. Sixteen of the 18 had received prior irradiation. None of the patients had received prior chemotherapy. Fifteen patients had previously received hormones without response. No patients received concurrent hormone treatments. Ten of the 18 patients (56%) achieved an objective response: five (28%) achieved complete response and five (28%) achieved partial response. Myelosuppression was present in seven patients, and two developed neurotoxicity. Combination chemotherapy using cisplatin, doxorubicin, and cyclophosphamide is active in patients with adenocarcinoma of the endometrium, including those who have received prior irradiation and have failed hormonal therapy. [Cancer Treat Rep 70:789–791, 1986.]

Previous chemotherapy reports from this institution have shown responses in advanced endometrial carcinoma to cisplatin (P) used as a single agent (1) or to the combination of doxorubicin (A) and cyclophosphamide (C) (2). The present study was undertaken to determine the rate of response and the toxic effects from the combination of PAC in patients with metastatic or recurrent endometrial cancer at The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston.

MATERIALS AND METHODS

Eighteen patients with adenocarcinoma of the endometrium were treated with PAC between January 1981 and December 1983. No other therapy was administered concurrently and no patient had received prior chemotherapy. Patients were considered eligible for the treatment if they had developed recurrent disease after initial treatment with surgery and/or radiation therapy or were found to have extensive disease at the time of initial surgery. All patients had evaluable disease, measurable either radiographically or by clinical examination. Informed consent was obtained under an institutional review board-approved protocol.

All but one patient received at least two courses of chemotherapy. Tumors were graded histologically, and patients were staged according to the FIGO (International Federation of Gynecology and Obstetrics) classification for endometrial cancer. A *complete response* was noted when all known existing disease disappeared for at least 1 month. A *partial response* was defined as a reduction of $\geq 50\%$ in the product of the two greatest diameters of measurable lesions for a minimum of 1 month. When no change occurred or when less than a partial response was seen over an 8-week period, the disease was considered *stable*. An increase of $> 25\%$ in existing lesions or the appearance of any new lesions indicated *disease progression*.

Patients were examined every 4 weeks; laboratory studies included urinalysis, renal function tests, cbcs, chest radiograph, and serial electrocardiograms and cardiac ejection fractions determined by isotope scan.

Cisplatin, at a dose of 50 mg/m^2 in 1 L of 0.45 normal saline with 50 g of mannitol added, was administered iv over 4 hours. Doxorubicin at a dose of 50 mg/m^2 and cyclophosphamide at a dose of 500 mg/m^2 were administered iv as a single bolus. In patients who had received prior pelvic irradiation, the starting doses were reduced to: cisplatin, 40 mg/m^2; doxorubicin, 40 mg/m^2; and cyclophosphamide, 400 mg/m^2. The doxorubicin was discontinued when a cumulative dose of 450 mg/m^2 was attained.

[1]Received July 18, 1985; revised Jan 10, 1986; accepted Jan 22, 1986.

[2]Department of Gynecology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[3]*Present address:* Department of Gynecology, Wilford Hall U.S. Air Force Medical Center, Lackland Air Force Base, TX.

[*]*Reprint requests to:* Ralph S. Freedman, MD, PhD, Department of Gynecology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Characteristics of responders

Age (yrs)	Stage/ grade	Prior treatment*	Site of recurrence	Response	No. of courses	Survival (mos)†
71	IB/III	R, WP, P	Lung	Complete	12	31
63	Unknown/III	R, T, P	Liver	Complete	12	26+
66	Unknown/Unknown	R, WP, T	Lung	Complete	10	14
61	II/III	R, WP, P	Abdomen	Complete	15	31
54	IB/III	R, WP, P	Abdomen	Complete	11	18
60	IB/II	R, WP, P, T	Abdomen	Partial	5	11
68	II/II	R, P, T	Lung/abdomen	Partial	6	11
57	IV/II	WP, P, T	Abdomen	Partial	12	14
61	IV/III	—	Lung/abdomen	Partial	8	23+
49	IV/III	R	Abdomen/liver	Partial	6	10

*R = radium; WP = whole-pelvis irradiation; P = progestin; and T = tamoxifen.
†From initiation of chemotherapy.

RESULTS (table 1)

All patients were evaluated. Ten of 18 patients (56%) achieved an objective response: five (28%) were complete responders and five (28%) were partial responders. There were no patients in this series with grade I tumors. Hormone receptor data were not available in the majority of patients, since their initial surgery was usually performed elsewhere. Fifteen of 18 patients had received prior hormonal therapy. Five patients had been treated with progestins, two with tamoxifen, and eight with a combination of tamoxifen and progestins. Sixteen of 18 patients had received prior interstitial and/or whole-pelvis irradiation: five patients were treated with intracavitary radium only and 11 patients were treated with intracavitary radium and whole-pelvis irradiation. The type and amount of prior radiotherapy did not appear to influence the response rate. The ages of the patients ranged from 46 to 78 years (mean, 57.6). All patients had a Karnofsky performance status > 80%.

Complete responders received 10–12 courses of PAC therapy. Of the five patients showing a complete response, four were clinically free of disease after only three cycles of chemotherapy, while one patient required six cycles.

All patients experienced alopecia, nausea, and vomiting. Neutropenia, with total granulocyte counts of $< 1000/mm^3$, was experienced by seven patients (39%). Five patients (28%) required dose reductions because of myelosuppression. Three patients developed severe nausea and vomiting, one discontinuing therapy after two cycles of chemotherapy. No renal or cardiac abnormalities were detected that resulted in discontinuation of therapy. Two patients developed neurotoxicity secondary to cisplatin, one with ototoxicity after 11 courses and one with peripheral neuropathy after ten courses. One doxorubicin extravasation occurred that resolved with conservative therapy. All but one patient received chemotherapy as outpatients. No hospitalizations for sepsis were necessary and no treatment deaths occurred.

DISCUSSION

The response rate to cisplatin as a single agent in advanced or recurrent adenocarcinoma of the endometrium ranges from 4% to 32% (2,3). The Gynecology Oncology Group had only one response (4%) among 23 patients, but 20 of 23 patients in that study had previously received doxorubicin or an alkylating agent. The rate of response previously reported from M. D. Anderson Hospital for the combination of doxorubicin and cyclophosphamide is 31%. Muggia et al (4) have reported a 45.5% response in 11 patients who received this combination.

The response rate of 56% (complete response, 28%) with PAC is similar to the 47% response rate reported by Turbow et al (5) and the 60% response rate reported by Lovecchio et al (6), although a progestational agent had been included in the latter regimen. Pasmantier et al (7) recently reported a 92% response rate with the combination of doxorubicin and cisplatin (median survival, 10 months), and they consider that the higher response rate could be due to the higher doses of cisplatin and doxorubicin that could be administered by omitting cyclophosphamide. Thigpen et al (8) have reported that the addition of cyclophosphamide to doxorubicin was no better than doxorubicin alone.

Our findings indicate that PAC is an active combination for the treatment of patients with advanced endometrial cancer and that responses are apparent usually within two courses. Toxicity was of moderate severity but was reasonably tolerated by this patient population. Further studies comparing PAC with PA or other combinations should be considered.

REFERENCES

1. SESKI JC, EDWARDS CL, GERSHENSON DM, ET AL. Doxorubicin and cyclophosphamide chemotherapy for disseminated endometrial cancer. Obstet Gynecol 58:88–91, 1981.
2. SESKI JC, EDWARDS CL, HERSON J, ET AL. Cisplatin chemotherapy for disseminated endometrial cancer. Obstet Gynecol 59:225–228, 1982.

3. THIGPEN JT, BLESSING JA, LAGASSE LD, ET AL. Phase II trial of cisplatin as second-line chemotherapy in patients with advanced or recurrent endometrial carcinoma. Am J Clin Oncol 7:253–256, 1984.
4. MUGGIA GM, CHIA G, REED LJ, ET AL. Doxorubicin-cyclophosphamide: effective chemotherapy for advanced endometrial adenocarcinoma. Am J Obstet Gynecol 128:314–319, 1977.
5. TURBOW MM, BALLON SC, SIKIC BL, ET AL. Cisplatin, doxorubicin, and cyclophosphamide chemotherapy for advanced endometrial carcinoma. Cancer Treat Rep 69:465–467, 1985.
6. LOVECCHIO JL, AVERETTE HE, LICHTINGER M, ET AL. Treatment of advanced or recurrent endometrial adenocarcinoma with cyclophosphamide, doxorubicin, cis-platinum, and megestrol acetate. Obstet Gynecol 63:557–560, 1984.
7. PASMANTIER MW, COLEMAN M, SILVER RT, ET AL. Treatment of advanced endometrial carcinoma with doxorubicin and cisplatin: effects on both untreated and previously treated patients. Cancer Treat Rep 69:539–542, 1985.
8. THIGPEN J, BLESSING J, DISAIA P, ET AL. A randomized trial of adriamycin with or without cyclophosphamide in the treatment of advanced or recurrent endometrial carcinoma. Proc ASCO 4:115, 1985.

Phase II Study of the Platinum Analog TNO-6 in Patients With Advanced Ovarian Cancer[1,2]

Nicoletta Colombo,[3,*] Enrico Sartori,[4] Fabio Landoni,[3] Giuseppe Favalli,[4] Laura Vassena,[3] Lucia Zotti,[4] Eric Materman,[5] Christopher R. Franks,[5] Sergio Pecorelli,[4] and Costantino Mangioni[3]

TNO-6 [*cis*-1,1-diaminomethylcyclohexane platinum (II) sulfate] is a second-generation platinum analog, lacking cross-resistance with cisplatin in animal models (1). Since a favorable toxicity profile emerged from preclinical studies (1,2) and a phase I study (3), TNO-6 was considered to be a useful candidate as second-line therapy in patients with advanced ovarian cancer pretreated with cisplatin.

PATIENTS AND METHODS

Thirty-eight patients with histologically proven advanced ovarian cancer were entered in this study (table 1), 30 of whom were evaluable for response and toxicity. Twenty-nine patients had received multiple courses of single-agent cisplatin; cisplatin and cyclophosphamide; or cisplatin, cyclophosphamide, and doxorubicin. One patient did not receive cisplatin. On entry in the study, patients had measurable disease, were fully recovered from previous therapy, and had wbc and platelet counts > 4000 and $100{,}000/mm^3$, respectively. Liver function tests were within normal range and creatinine clearance was > 50 ml/minute. Monthly examinations included audiometry, chest x-ray, hematology, liver and renal function tests (including renogram ^{123}I), urinalysis, and plasma electrolytes. Hematology and limited renal function tests were repeated weekly.

TNO-6 was given as an iv infusion over 15 minutes every 4 weeks at a starting dose of 30 mg/m^2. Dose modifications of ± 5 mg/m^2 were made according to platelet and wbc count nadirs. Twenty-six patients received TNO-6 without hydration; four received hydration during the second and subsequent courses. Patients were evaluable for response and toxicity 28 days after the first dose.

TABLE 1.—Patient characteristics and toxicity

Characteristic	No. of patients
Entered	38
Evaluable for response and toxicity	30
Median age in yrs (range): 54 (32-69)	
Median Karnofsky performance status (range): 100 (60-100)	
No. of courses received: 72	
Median No. of courses (range): 2 (1-6)	
Hematologic toxicity ($\times 10^9$ cells/L)	
Wbc count nadir	
3	22
2-2.9	7
1-1.9	1
< 1	0
Platelet count nadir	
100	24
50-99	4
25-49	2
< 25	0
Renal toxicity*	
BUN	
Grade 0	26
Grade 1	3
Grade 2	1†
Serum creatinine (no test in 2 patients)	
Grade 0	27
Grade 1	1
Grade 2	1†

*World Health Organization scale.

†During the 6th cycle *s*-creatinine rose in this patient to 4.7 mg/100 ml and BUN rose to 78 mg/100 ml; 3 mos later, *s*-creatinine was 15.4 mg/100 ml. 2 additional patients, who developed acute renal failure 6 days after the 1st course, are not included since they are inevaluable.

RESULTS

None of the 30 patients evaluable for response and toxicity achieved remission. Renal toxicity was generally mild (table 1), except in one patient who completed six cycles of TNO-6. Renal function gradually deteriorated from the fourth and subsequent cycles. In addition, two

[1]Received May 8, 1985; revised Nov 7, 1985; accepted Dec 2, 1985.

[2]Supported by a grant from Bristol-Myers Pharmaceutical Research and Development, Brussels, Belgium.

[3]Clinica Ostetrica Ginecologica Ospedale S. Gerardo, Monza, Settore Didattico Universita di Milano, Italy.

[4]Clinica Ostetrica Ginecologica, Universita di Brescia, Brescia, Italy.

[5]Bristol-Myers Pharmaceutical Research and Development, Brussels, Belgium.

[*]*Reprint requests to:* Nicoletta Colombo, MD, Division of Oncology, New York University Medical Center, Old Bellevue Administration Bldg, Rm 224, 462 First Ave, New York, NY 10016.

patients with normal pretreatment renal function subsequently developed acute renal failure 6 days after the first dose. Neither one was evaluable for response or toxicity.

DISCUSSION

The lack of activity in 30 evaluable patients and the incidence of unpredictable severe renal toxicity suggest that TNO-6 may not have an advantage over cisplatin as a second-line agent in advanced ovarian cancer. In two broad phase II studies (4,5), TNO-6 showed minimal activity in a wide variety of solid tumors. In addition, eight of the 274 patients treated developed severe nephrotoxicity.

REFERENCES

1. ROSE WC, SCHURIG JE, HUFFALEN JB, ET AL. Antitumor activity and toxicity of cisplatin analogs. Cancer Treat Rep 66:135–146, 1982.
2. LELIEVELD P, and VAN PUTTEN LM. Preclinical investigations with the new platinum compound TNO-6. *In* Proceedings of the 12th International Congress of Chemotherapy 60, vol 12, 1981.
3. PINEDO H, TEN BOKKEL-HUININCK W, SIMONETT G, ET AL. Phase I study and pharmacokinetics of *cis*-1, 1-di(amino-methyl)-cyclohexane Pt II sulfate (TNO-6). Proc ASCO 1:32, 1982.
4. FRANKS CR, NYS G, MATERMAN E, ET AL. TNO-6 (1, 1-diaminomethyl-cyclohexane sulphate platinum II, NSL311056) in phase II trials. Dev Oncol 17:257, 1984.
5. VERMORKEN J, TEN BOKKEL-HUININCK W, SLEYFER DT, ET AL. Phase II study of 1, 1-diaminomethylcyclohexane sulphatoplatinum II (TNO-6). NCI-EORTC meeting, Brussels, No. 35, 1983.

Combination Chemotherapy With Vindesine and Cisplatin for Refractory Small Cell Bronchogenic Carcinoma[1,2]

Delia F. Chiuten,[3,*] David T. Carr,[3] Hari M. Dhingra,[3] William K. Murphy,[3] Gary Spitzer,[3] Theera Umsawasdi,[3] Gerald P. Bodey,[3] and Manuel Valdivieso[4,5]

Vindesine has shown clinical activity against small cell bronchogenic carcinoma (SCBC) (1,2). In phase II trials, vindesine by single bolus injection for refractory SCBC yielded a 21%-27% response rate (median duration, 1-2 months) (1,2). Single-agent cisplatin yields a 6%-22% response rate in SCBC (3-4). We report a phase II trial of vindesine and cisplatin for previously treated SCBC.

PATIENTS AND METHODS

Twenty-two patients with locally advanced and metastatic SCBC (all stage III) refractory to standard combination chemotherapy including cyclophosphamide, doxorubicin, vincristine, and etoposide were evaluated (table 1). Vindesine was injected daily at 0.4 mg/m^2 for 5 days, followed by cisplatin at 60 mg/m^2 as a 4-hour infusion in 1000 ml of 5% dextrose in water and one-half normal saline or normal saline and 250 ml of 20% mannitol on Day 6. Dose was reduced by 25% for absolute granulocyte count $< 1000/mm^3$ and platelet count $< 75,000/mm^3$. Courses were repeated every 3 weeks. The treatment median was two courses (range, one to seven). Physical examinations, routine tests (eg, hematologic and biochemical parameters), and chest roentgenographs were done before treatment and during each course. Standard response criteria were used.

RESULTS

Four patients who had received prior vincristine responded; the response rate was 18% with a 95% confidence limit (range, 2%-34%). One response was complete (duration, 6 months; survival, 8 months); three were partial (duration, 3.5, 7, and 7 months, respectively; survival range, 3.5-10 months). The overall median duration of response was 6.5 months (range, 3.5-7). Eighteen patients had progressive disease; their median survival was 2.5 months (range, 1-3.5).

Myelosuppression was moderate. One patient developed hematuria and four required dose reductions. Table 1 shows other toxic effects.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	22
Median age in yrs (range): 57 (42-72)	
Performance status*	
1	4
2	6
3	6
4	1
Unknown	5
Previous treatment	
Chemotherapy	22
Radiotherapy	
Chest	6
Brain	11
Extent of disease	
Limited	3
Extensive	19
Toxic effects (% of courses)	
Neutropenia† (< 1000 cells/mm^3): 35	
Thrombocytopenia (< 50,000 cells/mm^3): 17	
Nausea and vomiting: universal	
Paresthesia: 14	
Anorexia: 7	
Fluid overload: 5	
Weakness: 4	
Constipation: 4	
Diarrhea: 2	

*Zubrod scale.
†Associated with 4 episodes of sepsis.

[1]Received May 13, 1985; revised Nov 4, 1985; accepted Dec 4, 1985.
[2]Supported in part by Public Health Service grant CA-05831 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Division of Medicine, Departments of Internal Medicine and Medical Oncology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.
[4]*Present address:* Department of Medical Oncology, American International Hospital, Zion, IL.
[5]We thank Dr. Barry Brown of the Department of Biomathematics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, for statistical help.
Reprint requests to: Dr. Delia F. Chiuten, Division of Medicine, Department of Medical Oncology, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

DISCUSSION

Our response rate is comparable to the 19% of other clinical trials using higher doses (vindesine, 3–4 mg/m^2; cisplatin, 60–100 mg/m^2) in refractory SCBC (5). Peripheral neuropathy, manifested mainly as paresthesia, was cumulative after two to three courses. Paresthesia incidence (14%) was higher than in our previous studies (6) using vindesine alone at 2–6 mg/m^2 once every 2 weeks (6%). In terms of response, the present regimen had modest activity in previously treated SCBC.

REFERENCES

1. NATALE RB, GRALLA RJ, and WITTES RE. Phase II trial of vindesine in patients with small cell lung carcinoma. Cancer Treat Rep 65:129–131, 1981.
2. ØSTERLIND K, DOMBERNOWSKY P, SØRENSEN PG, ET AL. Vindesine in the treatment of small cell anaplastic bronchogenic carcinoma. Cancer Treat Rep 65:245–248, 1981.
3. CAVALLI F, GOLDHIRSH A, SIEGENTHALER P, ET AL. Phase II study with *cis*-dichlorodiammineplatinum (II) in small cell anaplastic bronchogenic carcinoma. Eur J Cancer 16:620–628, 1980.
4. LEVENSON RM, JR, IHDE DC, HUBERMAN MS, ET AL. Phase II trial of cisplatin in small cell carcinoma of the lung. Cancer Treat Rep 65:905–907, 1981.
5. NEIDERLE N, SCHÜTTE J, SCHMIDT CG, ET AL. Treatment of recurrent small cell lung carcinoma with vindesine and cisplatin. Cancer Treat Rep 68:791–792, 1984.
6. VALDIVIESO M. Phase I and II studies of vindesine. Cancer Treat Rev 7(suppl):31–37, 1980.

Abbreviated Phase I Clinical Trial of Iv Mitozolomide[1,2,3]

R. A. Joss,* H. J. Ryssel, A. K. Bischoff, A. Goldhirsch, and K. W. Brunner[4]

Newlands et al (1) reported in the July/August 1985 issue of *Cancer Treatment Reports* their results of a phase I trial with mitozolomide (previously azolastone). Since figure 1 of Dr. Newlands' article was omitted, the structural formula of mitozolomide is included in this paper. In their trial, Newlands et al found only minimal hematologic toxicity at a dose of 115 mg/m^2 iv [median wbc count nadir, 4300/mm^3 (range, 1500-8200) and median platelet count nadir, 116,000/mm^3 (range, 52,000-208,000)], whereas myelosuppression was rather severe at 125 mg/m^2 [median wbc count nadir, 2500/mm^3 (range, 1200-6800) and median platelet count nadir, 21,000/mm^3 (range, 7000-75,000)]. When the European Organization for Research on Treatment of Cancer-Early Clinical Trials Group was planning future phase II trials with mitozolomide, Dr. Newlands kindly provided the Group with his results. The Early Clinical Trials Group decided to perform an abbreviated phase I trial before embarking on phase II studies. Since different investigators reported unexpected toxicity in early phase II trials at an iv dose of 115 mg/m^2, we first explored a level of 100 mg/m^2 and later reduced it to 90 mg/m^2.

Mitozolomide was provided as a lyophilized powder in vials containing 200 mg of the drug. Dimethyl sulfoxide (5 ml) was transferred into the vial containing the mitozolomide and after thorough dissolution the prescribed amount of drug was added to 500 ml of normal saline in a polyethylene infusion bag. Mitozolomide was administered as a 1-hour infusion. No antiemetics were used during the first course of the drug.

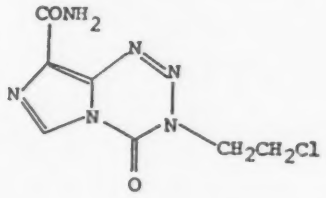


FIGURE 1.—Structure of mitozolomide.

A total of 11 patients (nine males and two females), with a median age of 50 years (range, 33-71), were entered in the trial. One patient had a performance status (PS) [World Health Organization (WHO) scale] of 0, seven had a PS of 1, and three had a PS of 2. Four patients had non-small cell lung carcinoma, one had gastric cancer, four had colorectal carcinoma, and one each had melanoma and soft tissue sarcoma. One patient had received prior radiotherapy, four had received prior radiotherapy and chemotherapy, and six had had prior chemotherapy. Hematologic toxic effects are shown in table 1.

Except for the dose-limiting myelosuppression, with thrombocytopenia being more pronounced than leuko-

TABLE 1.—Hematologic toxic effects

Dose (mg/m^2)	No. of patients/ No. of courses	Median nadir count, × 10^9 cells/L (range)					
		Wbc	Day of nadir	Day of recovery	Platelet	Day of nadir	Day of recovery
100	6/8	2.2 (1.3-3.9)	42 (24-46)	49 (31-71)	54 (27-113)	29 (17-29)	36 (24-42)
90	7/9	3.5 (2.6-8.3)	41 (15-65)	*	80 (30-156)	34 (20-43)	43 (30-61)

* 22, 32, 61, and 79 days; value not assessable in 2 patients with wbc count nadir < 4000/mm^3.

[1]Received Nov 18, 1985; accepted Dec 5, 1985.

[2]This trial was performed within the framework of the European Organization for Research on Treatment of Cancer-Early Clinical Trials Group (Chairman, H. M. Pinedo).

[3]Mitozolomide was supplied by Dr. F. Djebbar, Rhône-Poulenc Santé, Courbevoie, France.

[4]Institute for Medical Oncology (*R. A. Joss, H. J. Ryssel, A. K. Bischoff,* and *K. W. Brunner*), and Ludwig Institute for Cancer Research (*A. Goldhirsch*), University of Bern, Inselspital, Switzerland.

* *Reprint requests to:* Dr. R. A. Joss, Institute of Medical Oncology, University of Bern, Inselspital, CH-3010 Bern, Switzerland.

penia, the other toxic effects observed were mild (table 1). During four courses, three patients experienced no nausea and vomiting, whereas seven had mild to moderate (WHO grade 1-2) gastrointestinal upset during a total of 11 courses and one patient had severe (WHO grade 3) vomiting during one course and mild gastrointestinal disturbance during another. No antitumor activity was observed in our trial.

Based on our results we would recommend an iv dose of mitozolomide of 100 mg/m^2 for previously untreated and 90 mg/m^2 for previously treated patients in phase II trials.

REFERENCE

1. NEWLANDS ES, BLACKLEDGE G, SLACK JA, ET AL. Phase I clinical trial of mitozolomide. Cancer Treat Rep 69:801-805, 1985.

Phase II Trial of Aclarubicin in Epidermoid Carcinoma of the Head and Neck[1,2]

Alejo A. Carugati,[3,*] Americo J. Olivari,[3] Daniel M. Campos,[3] Roberto Pradier,[3] and Paul V. Woolley[4]

We report here a phase II cross-over study using aclarubicin and methotrexate as sequential treatment in epidermoid carcinoma of the head and neck.

MATERIALS AND METHODS

Patients eligible for study had measurable epidermoid carcinoma of the head and neck. Those patients who had not received prior chemotherapy were randomly allotted to receive either aclarubicin [80 mg/m^2 every 4 weeks (1,2)] or methotrexate (40 mg/m^2/week). Aclarubicin was escalated to 100 mg/m^2 if toxicity in the first course was acceptable, ie, platelet count $\geq$ 100,000/mm^3 and wbc count $\geq$ 2500/mm^3. Patients were crossed over to the other drug at disease progression. Those patients who had received prior chemotherapy at the time of entering the protocol received aclarubicin only.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	27
Sex	
Male	4
Female	23
Median age in yrs (range): 53 (34-71)	
Median performance status (range):* 2 (1-3)	
Site of original disease	
Oral cavity	11
Pharynx	10
Larynx	4
Pyriform sinus	2
Prior therapy	
None	3
Radiation therapy only	20
Chemotherapy only	0
Radiation therapy + chemotherapy	4

* Eastern Cooperative Oncology Group scale.

RESULTS

Twenty-seven patients were entered in this study (tables 1 and 2), of whom four had received prior chemotherapy and 23 had not. Twelve of the latter 23 patients began treatment with aclarubicin and 11 started treatment with methotrexate. Eight patients received one course of aclarubicin, ten received two courses, and two received three courses. Six of the 12 patients who received more than one course were escalated to 100 mg/m^2 after the first course. No patient started on aclarubicin responded to treatment. One patient was lost to follow-up. Another patient died 6 days after receiving the drug, but was recorded as a failure to respond. Five of the remaining ten patients crossed-over to methotrexate and two had objective responses lasting 5 and 7 months. Eleven patients began treatment with methotrexate. One patient was lost to follow-up, two had partial response and received aclarubicin at disease progression, and one had complete response and never received aclarubicin. Five patients received aclarubicin after methotrexate and none had objective response. In summary, 20 evaluable patients were treated with aclarubicin and none showed evidence of objective response.

Aclarubicin produced moderate leukopenia, vomiting, diarrhea, and mucositis. One patient had a wbc count nadir of 1300/mm^3, ten had nadirs between 3000 and 4000/mm^3, and the remainder had no recorded wbc count nadir. One patient developed reversible elevation of serum transaminases. Seven patients treated with aclarubicin had initial and follow-up evaluation of left ventricular ejection fraction, and none showed a change to an abnormal value or developed clinical congestive heart failure.

[1]Received Jan 2, 1985; revised Dec 16, 1985; accepted Dec 23, 1985.

[2]This protocol was conducted as a collaboration between the Instituto de Oncologia "Angel H. Roffo," University of Buenos Aires, and the Division of Medical Oncology, Georgetown University, under the auspices of the National Cancer Institute-Pan American Health Organization Collaborative Cancer Treatment Research Program contract N01-CO-65332 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Instituto de Oncologia "Angel H. Roffo," University of Buenos Aires, Argentina.

[4]Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC.

*_Reprint requests to:_ Paul V. Woolley, MD, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 2.—Response data by treatment*

	Total	Objective response	Crossed over to opposite arm after failure of initial therapy	Response to cross-over
No prior chemotherapy				
Initial aclarubicin treatment				
Evaluable	11	0	5	2 to methotrexate
Inevaluable	1			
Subtotal	12			
Initial methotrexate treatment				
Evaluable	10	3†	5	0 to aclarubicin
Inevaluable	1			
Subtotal	11			
Prior chemotherapy				
Aclarubicin treatment	4	0	—	
Total	27			

*Values = No. of patients.
†One complete response and 2 partial responses.

DISCUSSION

None of the 20 patients treated with aclarubicin had objective disease response. The 95% one-sided confidence interval for 20 consecutive nonresponding patients ranges from 0% to 14%. If only the 11 patients who had had no prior chemotherapy are considered, the response could be as high as 24% using a one-sided confidence interval and 28.5% using a two-sided interval. The failure to respond to aclarubicin was not the result of an intrinsic drug resistance of the entire population, since five of 15 patients treated responded to methotrexate. Several patients who were treated with aclarubicin as initial therapy showed rapid disease progression. While more than one course of drug would be desirable during its phase II evaluation in a particular patient, these progressions were taken as evidence of lack of efficacy of the aclarubicin even on its first course. These data do not encourage the use of aclarubicin in head and neck cancer.

REFERENCES

1. WOOLLEY PV, AYOOB MJ, LEVENSON SM, ET AL. A phase I clinical trial of aclacinomycin-A administered on a five-consecutive-day schedule. J Clin Pharm 22:359-365, 1982.
2. CASPER ES, GRALLA RJ, and YOUNG CW. Clinical phase I study of aclacinomycin-A by evaluation of an intermittent intravenous schedule. Cancer Res 41:2417-2420, 1981.

Phase II Evaluation of Etoposide in Refractory Multiple Myeloma: A Southeastern Cancer Study Group Trial[1,2]

J. P. Gockerman,[3,*] A. A. Bartolucci,[4] M. O. Nelson,[5] H. Silberman,[3] E. Velez-Garcia,[6] and R. Stein[7]

Etoposide is a semisynthetic derivative of podophyllotoxin whose mechanism of action appears to be related to inhibition of the enzyme topoisomerase (1,2). The dose-limiting toxic effect is myelotoxicity. The agent has been useful in treating a number of human tumors but there has been no formal evaluation of its use in multiple myeloma (3). We have evaluated etoposide as a single agent in the treatment of refractory multiple myeloma.

PATIENTS AND METHODS

Patients eligible for this study had a confirmed diagnosis of multiple myeloma based on standard criteria and had a measurable protein abnormality or a measurable plasmacytoma (4). All patients had been heavily pretreated and at least 4 weeks had elapsed from previous chemotherapy. Patients with cytopenias due to their myeloma were eligible. Written informed consent was required. Patients were stratified according to good- and poor-risk groups based on hemoglobin (> 9 g/100 ml), BUN (< 30 mg/100 ml), and calcium (< 12 mg/100 ml) for the good-risk group (5). To be fully evaluable, patients must have received etoposide once every 3 weeks for four complete cycles. Patients were evaluated for survival and toxicity if they had received at least one cycle of etoposide. The starting dose was 50 mg/m^2 × 5 days in patients with good marrow reserve (wbc count > 4500/mm^3; absolute granulocyte count > 1900/mm^3; platelet count ⩾ 130,000/mm^3; and hemoglobin ⩾ 11.0 g/100 ml) or 40 mg/m^2 × 5 days in other patients with poor marrow reserve. The abnormal protein component or plasmacytoma was followed to measure disease response using standard criteria (5).

RESULTS

Patient characteristics, toxicity, and treatment results are shown in table 1. Twenty of the 45 poor-risk and 28 of the 40 good-risk patients were fully evaluable. In the poor-risk group there were nine patients with major protocol violations, two with missing data, one with early death, four who were removed because of drug toxicity, and nine who died prior to four cycles of therapy. In the good-risk group there were seven patients with major protocol violations, two who refused further therapy, and three who died prior to four cycles of therapy. The poor-risk patients received less drug and serious hematologic toxicity was no greater than that of good-risk patients. Mild nausea, vomiting, and alopecia were seen. There were no clear differences in the amount of prior treatment between the two groups. There were early deaths associated with far-advanced disease in association with neutropenia from the etoposide. The starting dose of etoposide appears to be appropriate for patients with normal blood cell counts at the time of initial therapy with etoposide. There were no grade III or IV toxic effects other than hematologic, which could be ascribed to etoposide.

There were two partial responses and nine patients with no disease progression in the group of 48 fully evaluable patients. This result suggests that etoposide as a single agent is not useful in heavily pretreated multiple myeloma. Whether etoposide is active earlier in the course of the disease needs further study.

[1]Received Aug 1, 1985; revised Dec 9, 1985; accepted Dec 23, 1985.
[2]Supported in part by Public Health Service grants CA-24456, CA-19657, CA-03013, CA-03127, CA-12223, CA-23909, CA-28143, CA-03376, CA-07961, CA-20255, CA-15584, CA-17214, CA-27587, CA-03227, CA-05641, CA-17027, and CA-28138 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Duke University Medical Center, Durham, NC.
[4]University of Alabama, Birmingham.
[5]University of Kentucky, Lexington.
[6]University of Puerto Rico, San Juan.
[7]Vanderbilt University Medical Center, Nashville, TN.
[*] *Reprint requests to:* Jon P. Gockerman, MD, Box 3877, Duke University Medical Center, Durham, NC 27710.

TABLE 1.—Patient characteristics, toxicity, and treatment results*

	Patients	
	Poor-risk	Good-risk
Total		
Evaluable	45	40
Survival only	12	7
Toxicity only	13	5
Fully	20	28
Male/female	19/26	26/14
Median age (yrs)	62	60
Karnofsky performance status (%)		
30–50	2	16
60–70	15	15
80–90	16	6
100	2	1
Unknown	5	7
Median time from diagnosis to on study (mos)	30	28
% of full dose (4 courses) actually received		
25	19	5
50	4	5
75	3	9
100	19	19
125	—	1
150	—	1
Duration of treatment (wks)		
3	19	8
3–6	6	5
6–9	1	8
9–12	9	7
12	10	12
Toxicity (Grade III and IV)		
Neutropenia	16	16
Thrombocytopenia	16	0
Infection	2	2
Stomatitis	1	0
Response/time to disease progression after 3-mo evaluation (mos)		
Good partial response	0	1†
Fair partial response	0	1‡
No disease progression	4§	5¶
Disease progression	15	19
Died on study	1	2
Prior treatment**		
MP	3	3
MP, Vcr	1	1
MP, Cy	1	1
MP, Cy, Vcr, P	1	2
MP, Cy, Adr-Vcr	4	—
MP, BCP	9	13
MP, BCP, Adr-Vcr	10	7
MP, Adr-Vcr	1	2
MP, BCP, Vcr	1	1
MP, BCP, Vcr, CAP	1	1
MP, Pyr, Hxm	1	—
BCP	4	3
BCP, Adr-Vcr	1	3
No data	7	3

* Unless otherwise specified, values = No. of patients.

† 11.6 mos.

‡ 2 mos.

§ 1, 2.3, 7.8, and 18 mos.

¶ 2.6, 2.7, 3.1, 4.0, and 9.0 mos.

** MP = melphalan and prednisone; Vcr = vincristine; Cy = cyclophosphamide; P = prednisone; Adr-Vcr = doxorubicin and vincristine; BCP = carmustine, cyclophosphamide, and prednisone; CAP = cyclophosphamide, doxorubicin, and prednisone; Pyr = pyrazofurin; and Hxm = hexamethylmelamine.

REFERENCES

1. ISSELL BF, and CROOKE ST. Etoposide (VP-16-213). Cancer Treat Rev 6:107–124, 1979.
2. CAVALLI F. VP-16213 (etoposide): a critical review of its activity. Cancer Chemother Pharmacol 7:81–110, 1982.
3. BAGSHAWE K. VP-16. Recent advances and future prospects. Semin Oncol 12(suppl 2):81–85, 1985.
4. Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute. Proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep Part 3, vol 1(1):17–39, 1968.
5. COHEN HJ, SILBERMAN HR, LAUREN WE, ET AL. Combination chemotherapy with intermittent 1-3 bis (2-chloroethyl) 1-nitrosourea (BCNU), cyclophosphamide, and prednisone for multiple myeloma. Blood 54:824–836, 1979.

Phase II Evaluation of Aclarubicin in Lung Cancer: A Southeastern Cancer Study Group Trial[1,2]

Barnett S. Kramer,* Robert Birch, Jon P. Gockerman, Anthony Greco, and Karen Prestridge[2]

Aclarubicin is an anthracycline antibiotic with a preclinical antitumor spectrum similar to that of doxorubicin and daunorubicin (1). It achieves higher intracellular concentrations than does daunorubicin; unlike daunorubicin, it concentrates in the cytoplasm rather than the nucleus and inhibits RNA synthesis out of proportion to DNA synthesis (2). A golden hamster model demonstrated less cardiotoxicity and alopecia with aclarubicin compared to doxorubicin and daunorubicin (3). In addition, aclarubicin has demonstrated possible activity as a differentiating agent (4,5).

We report a phase II trial in patients with lung cancer fulfilling the following entry criteria: (*a*) histologically confirmed diagnosis; (*b*) at least one evaluable lesion; (*c*) Karnofsky performance status ≥ 60%; (*d*) incurability by surgery or irradiation; (*e*) normal ECG; (*f*) relapse after front-line chemotherapy in patients with small cell carcinoma; (*g*) recovery from previous myelosuppressive therapy; (*h*) no brain metastases; and (*i*) signed informed consent. Aclarubicin was given as an iv bolus infusion every 21 days until disease progression was documented. The dose was 100 mg/m^2 iv (75 mg/m^2 iv in the 13 patients who were > 70 years of age, had extensive irradiation, or had extensive bone disease).

Of 99 patients entered in the study, seven were found ineligible and were dropped from analysis for the following reasons: no evaluable disease (two patients); low performance status (two); abnormal baseline ECG (one); brain metastases at study entry (one); and registered too late (one). An additional three patients could not be analyzed because no follow-up data were provided by the treating physician. The remaining population was as follows: 21 patients with squamous carcinoma (16 with no prior chemotherapy); 26 with adenocarcinoma (11 with no prior therapy); 28 with large cell carcinoma (18 with no prior chemotherapy); and 14 with small cell carcinoma. There was only one partial response lasting 6 weeks in a patient with large cell type who had previously received irradiation but no chemotherapy. The 95% confidence intervals were 1%-13% for all previously untreated patients with non-small cell carcinoma and

TABLE 1.—Toxicity data for aclarubicin*

Toxic effect	No prior chemotherapy	Prior chemotherapy
Nadir granulocyte count (× 10^3 cells/mm^3)		
1.5-1.89	12	5
1.0-1.49	14	10
0.5-0.99	5	26
< 0.5	17	15
Nadir platelet count (× 10^3 cells/mm^3)		
90-129	12	12
50-89	5	5
25-49	—	5
< 25	—	2
Gastrointestinal		
Nausea (no vomiting)	24	12
Controllable vomiting	43	52
Intractable vomiting	10	5
Hospitalization required	2	—
ECG		
ST-T wave changes	3	7
Unifocal premature ventricular contractions	—	7
Multifocal premature ventricular contractions	3	—
Ventricular tachycardia	—	—

* Values = % of patients for whom data were available.

[1]Received Dec 17, 1985; accepted Dec 24, 1985.

[2]Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: The University of Florida, Gainesville (*B. S. Kramer,* CA-28143); Southeastern Cancer Study Group Statistical Center, University of Alabama in Birmingham (*R. Birch* and *K. Prestridge,* CA-24456); Vanderbilt University School of Medicine, Nashville, TN (*A. Greco,* CA-23909); and Duke University School of Medicine, Durham, NC (*J. P. Gockerman,* CA-03177). Other participating institutions include: Emory University, Atlanta, GA; Case Western University, Detroit, MI (CA-15584); University of Tennessee, Knoxville (CA-13237); Indiana University, Indianapolis (CA-28171); Washington University, St. Louis, MO (CA-03376); University of Puerto Rico, San Juan (CA-12223); St. Louis University, MO (CA-28169); Temple University, Philadelphia, PA (CA-07961); CCOP-Augusta, GA; University of Miami, FL; University of Kentucky, Louisville (CA-20255); University of Kentucky, Lexington (CA-20255); University of Cincinnati, OH (CA-28138); City of Hope, Duarte, CA; University of Virginia, Charlottesville; University of Iowa, Iowa City (CA-37577); East Carolina University, Greenville, NC; University of Tennessee, Memphis (CA-13237); University of South Florida, Tampa (CA-28143); and CCOP-Roanoke, VA.

* *Reprint requests to:* Barnett S. Kramer, MD, J. H. Miller Health Center, Box J-277, Gainesville, FL 32610.

0%-27% for patients with small cell carcinoma. Median survival for all eligible patients was 17 weeks.

Toxic effects are shown in table 1. Myelosuppression was dose-limiting, but there was modest gastrointestinal toxicity and occasional significant ECG changes.

In conclusion, aclarubicin had no significant activity in our population of patients with bronchogenic carcinoma with this dose schedule.

REFERENCES

1. HORI S, SHIRAI M, HIRANO S, ET AL. Antitumor activity of new anthracycline antibiotics, aclacinomycin-A and its analogs, and their toxicity. Gann 68:685-690, 1977.
2. EGORIN MJ, CLAWSON RE, ROSS LA, ET AL. Cellular accumulation and disposition of aclacinomycin-A. Cancer Res 39:4396-4400, 1979.
3. DANTCHEV D, BALERCIA G, BOURUT C, ET AL. Comparative microscopic study of cardiotoxicity and skin toxicity of anthracycline analogs. Biomed Pharmacother 38:322-328, 1984.
4. SCHWARTZ EL, BROWN BJ, NIERENBURG M, ET AL. Evaluation of some anthracycline antibiotics in an in vivo model for studying drug-induced human leukemia cell differentiation. Cancer Res 43:2725-2730, 1983.
5. SAKURAV M, SMAMPI K, and HOZUMI M. Possible differentiation of human acute myeloblastic leukemia cells by daily and intermittent administration of aclacinomycin A. Leuk Res 7:139-143, 1983.

Epirubicin in Non-Oat Cell Lung Cancer—Response Rates and the Importance of Immunopathology: A Northern California Oncology Group Study[1,2]

Frederick J. Meyers,[3,*] Robert D. Cardiff,[3] Robert Quadro,[3] Melanie Gribble,[4] Marsha Kohler,[4] Victor Medrano,[5] Edith P. Mitchell,[6] Roger Shiffman,[7] and Larry William[8]

Epirubicin is a derivative of doxorubicin which was synthesized with the aim of improving the spectrum of activity and of decreasing the toxic effects, specifically cardiac toxic effects (1). The cardiac toxic effects of epirubicin in patients with non-oat cell lung cancer form part of a larger Northern California Oncology Group study. Endomyocardial biopsy was used to assess cardiac toxic effects of epirubicin. This report summarizes the response rate of this agent in each of the three different non-oat cell histologies. Findings using modern techniques in immunopathology are described.

MATERIALS AND METHODS

Only patients considered incurable by surgery or irradiation were entered.

All patients had adequate renal, hepatic, and bone marrow functions (creatinine < 3.0 mg/dl, bilirubin < 3.0 mg/dl, wbc count > 4000/mm^3, and platelet count > 150,000/mm^3). Patients were excluded if they had a history of cardiac disease or had received prior chemotherapy. Signed informed consent was obtained from all patients.

The dose of epirubicin was 75 mg/m^2 iv over 5–10 minutes every 3 weeks. The next cycle was delayed 1 week for a wbc count < 3500/mm^3 or a platelet count < 100,000/mm^3. The dose was decreased by 25% after that 1 week for a wbc count of 3000–3499/mm^3 or a platelet count of 100,000–149,000/mm^3. Treatment of patients who responded or had stable disease was continued until disease progression. The slides of all responders were reviewed and immunoperoxidase studies were performed if adequate material was available.

RESULTS

Seventy-four patients were entered (table 1). Four patients were ineligible: one because a lung primary could not be histologically confirmed, one because radiation therapy was given within 3 weeks of study entry, and two because histologic review showed small cell lung cancer. Six patients were unevaluable, three never received therapy, two had no measurable disease, and one had no repeat measurements performed.

There were no responders in the squamous cell carcinoma group; one in the adenocarcinoma group; and three in the large cell undifferentiated group, including two complete responders.

Therapy was well-tolerated. Only one patient had a wbc count nadir < 1000/mm^3 without symptoms. This patient's dose was escalated for two consecutive cycles, in violation of the protocol. There were no serious (grade 3 or 4) nonhematologic toxic effects except for alopecia and two cases of grade 3 nausea and vomiting.

Immunopathology

Two of the four responders had sufficient fixed material available in paraffin blocks to perform immunoperoxidase studies. The method used was as previously described (2). Both were patients with large cell carcinoma.

The tumor of the first patient was positive for epithelial membrane antigen and negative for leukocyte common antigen and type 8 cytokeratin.

The tumor of the second patient was positive for LN-2 (3) and negative for alpha-fetoprotein, human chorionic gonadotropin, leukocyte common antigen, epithelial

[1]Received Aug 20, 1985; revised Dec 19, 1985; accepted Jan 6, 1986.

[2]Supported in part by Public Health Service grants CA-21744, CA-25838, CA-37457, CA-35011, and CA-39098 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of California School of Medicine, Davis.

[4]Northern California Oncology Group, Palo Alto.

[5]San Joaquin Valley Community Clinical Oncology Program, Fresno, CA.

[6]David Grant Medical Center, Travis Air Force Base, CA.

[7]Central Coast Cancer Council, Monterey, CA.

[8]South Bay Oncology Program, San Jose, CA.

[*] *Reprint requests to:* Frederick J. Meyers, MD, Division of Hematology and Oncology, Department of Internal Medicine, University of California School of Medicine, Davis, 4301 X St, Sacramento, CA 95817.

TABLE 1.—Patient characteristics and response to therapy

Characteristic	No. of patients (%)
Total	74
Not eligible	4
Not evaluable	6
Evaluable	64
Karnofsky performance status (%)	
70	27 (36)
80–100	47 (64)
Mean age in yrs (range): 59 (35–84)	
Sex	
Male	56
Female	18
Presence of distant metastases	
M0	36 (49)
M1	38 (51)
Response rate*	
Squamous cell carcinoma	0/19 (0)
Adenocarcinoma (1 + 0)	1/17 (6)
Large cell carcinoma (2 + 1)	3/28 (11)

* Complete + partial responses.

membrane antigen, type 8 cytokeratin, and LN-1. Based on these studies, we suggest that this patient has a non-Hodgkin's lymphoma.

DISCUSSION

This site-directed phase II trial shows no activity of epirubicin against advanced non-oat cell lung cancer. The drug was well-tolerated.

Special subtypes of non-oat cell carcinoma should be identified. Poorly differentiated extragonadal germ cell tumors may appear to be large cell carcinoma of lung (4). Further, poorly differentiated neoplasms that occur in the lung and mediastinum are not always epithelial in origin (5).

Immunopathology review of the tumor of all patients in non-oat cell clinical trials is unnecessary and expensive. However, immunopathology review of responding patients should be included in non-oat cell carcinoma therapy trials.

REFERENCES

1. ARACAMONE F, PENCO S, and VIGEVANI A. Adriamycin (NSC-123127): new chemical developments and analogs. Cancer Chemother Rep Part 3, vol 6(2):123–129, 1975.
2. MEYERS FJ, and GOODNIGHT JE. The identification of unsuspected thyroid carcinoma using immunoperoxidase for thyroglobulin. Am J Med (in press).
3. EPSTEIN AL, MARDER RJ, WINTER JN, ET AL. Two new monoclonal antibodies (LN-1, LN-2) reactive in B5 formalin-fixed, paraffin-embedded tissues with follicular center and mantle zone human B lymphocytes and derived tumors. J Immunol 133:1028–1036, 1984.
4. GRECO FA, OLDHAM RK, and FER MF. The extragonadal germ cell cancer syndrome. Semin Oncol 9:448–455, 1982.
5. BATTIFORA H. Recent progress in the immunohistochemistry of solid tumors. Semin Diag Pathol 1:251, 1984.

Phase II Study of Vindesine in the Treatment of Pediatric Patients With Solid Tumors: A Report From the Childrens Cancer Study Group[1,2]

Mark D. Krailo, William Krivit, Harland Sather, and Denman Hammond[2,*]

Vindesine (VND) was synthesized to provide an agent with less neurotoxicity and wider chemotherapeutic spectrum than vincristine or vinblastine. VND has demonstrated activity in patients with relapsed leukemia (1) and in a patient with refractory histiocytosis (1). These observations prompted a trial of VND in relapsed solid tumors by the Childrens Cancer Study Group.

Patients with relapsed solid tumors were treated with VND following physical examination, neurologic examination, blood cell counts, and renal and liver function tests. VND was administered as a 4 mg/m^2 bolus injection on a weekly basis. Index lesions were evaluated after three injections. Patients continued in the study until progressive disease or life-threatening toxicity was noted. Those patients exhibiting progressive disease before 3 weeks were considered nonresponders. Follow-up studies were repeated weekly.

Eighty-five children were entered in the study. Two patients received concurrent therapy and are considered for toxicity only. One patient with neuroblastoma achieved partial response. Seven patients demonstrated stable disease: three with osteogenic sarcoma (2-4 months); two with Wilms' tumor (3-4 months); one with rhabdomyosarcoma (2 months); and one with medulloblastoma (12

TABLE 1.—Results of weekly VND therapy for children with solid tumors

Diagnosis	No. of patients					
	Evaluable for response	Previously treated with vincristine or vinblastine	Complete response	Partial response	Stable disease	Progressive disease
Neuroblastoma*	16	16	0	1	1	14
Ewing's sarcoma†	12	12	0	0	0	12
Rhabdomyosarcoma/undifferentiated sarcoma†	12	11§	0	0	1	11
Osteogenic sarcoma‡	10	7	0	0	3	7
Wilms' tumor‡	10	9	0	0	2	8
Non-Hodgkin's lymphoma	8	7§	0	0	0	8
Malignant germ cell tumor	4	4	0	0	0	4
Hepatoma	3	2	0	0	0	3
Nasopharyngeal carcinoma	2	2	0	0	0	2
Histiocytosis	1	1	0	0	0	1
Hodgkin's disease	1	1	0	0	0	1
Medulloblastoma	1	1	0	0	1	0
Astrocytoma	1	1	0	0	0	1
Neurosarcoma	1	0§	0	0	0	1
Retinoblastoma	1	1	0	0	0	1
Total	83	75	0	1	8	73

*95% confidence interval for true response rate (partial response + complete response), 0.2%-30%.

†Significant evidence ($P < 0.10$) to contradict the hypothesis that VND evokes significant tumor reduction in at least 20% of patients in this category.

‡Marginal evidence ($0.10 < P < 0.15$) to contradict the hypothesis that VND evokes significant tumor reduction in at least 20% of patients in this category.

§1 patient in this disease category had unknown treatment history.

[1]Received June 10, 1985; revised Dec 3, 1985; accepted Jan 9, 1986.

[2]Contributing Childrens Cancer Study Group investigators, institutions, and grant Nos. are given in the Appendix. Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

Reprint requests to: Childrens Cancer Study Group, Operations Office, 199 N Lake Ave, Third Floor, Pasadena, CA 91101.

months). Objective measures showed no reduction in tumor mass for six of these patients; one patient with medulloblastoma showed a < 25% reduction in tumor mass. All patients exhibiting partial response or stable disease had previously received vincristine or vinblastine.

Thirty-nine patients had significant hematologic toxicity, principally anemia (hemoglobin < 7 mg/dl) or thrombocytopenia (platelet count < 50,000/mm^3). Twenty patients had significant neurologic toxicity, principally severe weakness, sensory loss, or debilitating pain in the extremities, lasting at least 3 weeks. Most toxic effects (92%) occurred by the third injection of VND. Table 1 shows the results of weekly VND therapy for children with solid tumors.

VND demonstrated no significant antitumor effect. Cross-resistance with vincristine, as seen in relapsed leukemia (2-5), may, in part, account for this observation. Ninety percent of the patients studied received vincristine as part of their initial therapy. This is common with most pediatric solid tumors. Significant hematologic and neurologic toxic effects were noted with the dose and schedule used. Overall, VND offers no advantage over other currently available vinca alkaloids in terms of response or toxicity.

APPENDIX

Principal Investigators of the Childrens Cancer Study Group

Institution	*Investigator*	*Grant No.*
Group Operations Office, University of Southern California Comprehensive Cancer Center, Los Angeles	Denman Hammond John Weiner Harland Sather Richard Sposto Mark Krailo Jonathan Buckley	CA-13539
University of Michigan Medical Center, Mott Children's Hospital, Ann Arbor	Ruth Heyn	CA-02971
University of California Medical Center, San Francisco	Arthur Ablin	CA-17829
University of Wisconsin Hospital, Madison	Nasrollah Shahidi	CA-05436
Children's Orthopedic Hospital and Medical Center, University of Washington, Seattle	Ronald Chard	CA-10382
Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH	Peter Coccia	CA-20320
Children's Hospital National Medical Center, George Washington University, Washington, DC	Sanford Leikin	CA-03888
Childrens Memorial Hospital, Northwestern, University, Chicago, IL	Helen Maurer	CA-07431
Children's Hospital of Los Angeles, University of Southern California	Jorge Ortega	CA-02649
Children's Hospital of Columbus, Ohio State University	Frederick Ruymann	CA-03750
Babies Hospital, Columbia University, New York, NY	Sergio Piomelli	CA-03526
Children's Hospital of Pittsburgh, University of Pittsburgh, PA	Vincent Albo	CA-36015
Vanderbilt University School of Medicine, Nashville, TN	John Lukens	CA-26270
Doernbecher Memorial Hospital for Children, University of Oregon Health Sciences, Portland	Robert Neerhout	CA-26044
University of Minnesota Health Sciences Center, Minneapolis	Mark Nesbit	CA-07306
University of Texas Health Sciences Center, San Antonio	Paul Zeltzer	CA-36004

Principal Investigators of the Childrens Cancer Study Group (cont'd)

Institution	*Investigator*	*Grant No.*
Memorial Sloan-Kettering Cancer Center, Cornell University, New York, NY	Peter Steinherz	—
James Whitcomb Riley Hospital for Children, Indiana University, Indianapolis	Robert Baehner	CA-13809
University of Utah Medical Center, Salt Lake City	Richard O'Brien	CA-10198
Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY	Denis R. Miller	CA-23742
Strong Memorial Hospital, University of Rochester, NY	Harvey Cohen	CA-11174
University of British Columbia, Vancouver, Canada	Mavis Teasdale	Vancouver Foundation grant; CA-29013
Children's Hospital Medical Center, University of Cincinnati, OH	Beatrice Lampkin	CA-26126
Harbor/University of California, Los Angeles and Miller Children's Medical Center, University of California, Los Angeles, Torrance, and Long Beach.	Jerry Finklestein	CA-14560
University of Iowa Hospitals and Clinic, Iowa City	Raymond Tannous	CA-29314
Children's Hospital of Denver, CO	David Tubergen	CA-28851
Izaak Walton Killam Hospital for Children, Halifax, Nova Scotia, Canada	Allan Pyesmany	Nova Scotia

REFERENCES

1. KRIVIT W, and HAMMOND D. Vindesine: a phase II study by the Childrens Cancer Study Group. Curr Chemother 1:1331-1334, 1978.
2. KRIVIT W, ANDERSON J, CHILCOTE R, ET AL. A study of the cross resistance of vincristine and vindesine in reinduction therapy for acute lymphocytic leukemia in relapse. Am J Pediatr Hematol Oncol 2:217-221, 1980.
3. ANDERSON J, KRIVIT W, THOMPSON J, ET AL. Cross-resistance of vincristine and vindesine in reinduction therapy for acute lymphocytic leukemia in relapse. Current Chemotherapy and Infectious Disease, *In* the Proceedings of 11th ICC and the 19th ICAAC, Amer Soc of Microbiol, 1567-1568, 1980.
4. KRIVIT W, ANDERSON J, CHILCOTE R, ET AL. Comparison of vincristine utilized simultaneously or 24 hours before cyclophosphamide in maintenance regimen of acute lymphoblastic leukemia in children. A report of the Childrens Cancer Study Group. Am J Pediatr Hematol Oncol 3:227-231, 1981.
5. ANDERSON J, KRIVIT W, CHILCOTE R, ET AL. Comparison of the therapeutic response of patients with childhood acute lymphoblastic leukemia in relapse to vindesine versus vincristine in combination with prednisone and L-asparaginase: a phase III trial. Cancer Treat Rep 65:1015-1019, 1981.

Tamoxifen in Advanced and Recurrent Uterine Sarcomas: A Phase II Study[1]

Gynecological Group, Clinical Oncological Society of Australia and The Cancer Institute, Melbourne, Australia[2,3,*]

Uterine sarcomas arise in tissue of Müllerian (paramesonephric) origin, which in the normal nonmalignant state is exquisitely sensitive to estrogen stimulation. A significant proportion of uterine sarcomas of all varieties contain estrogen receptors (ER) and progesterone receptors (PR) (1) (R. M. Rome and M. A. Quinn, unpublished data), suggesting that they may be hormonally sensitive. Furthermore, there have been numerous anecdotes of responses of uterine sarcomas to treatment with progestogens and antiestrogens. In 1982, the Gynecological Group of the Clinical Oncological Society of Australia initiated a phase II study of the antiestrogen tamoxifen in advanced and recurrent uterine sarcoma.

PATIENTS AND METHODS

Patients of any age with histologically proven and measurable advanced or recurrent uterine sarcoma were eligible. Prior treatment with chemotherapy did not preclude entry provided it had not been administered within the preceding 3 weeks. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3, none had received radiotherapy to the measurable lesion, none had received prior hormonal therapy, and each had given informed consent. Tamoxifen (Nolvadex) was given orally at a dose of 20 mg twice a day. Patients were assessed 4 and 8 weeks later. Those patients who showed no change or evidence of tumor response (2) continued receiving tamoxifen therapy until there was evidence of tumor progression. Those patients with unequivocal evidence of tumor progression went off the study. The histology of each sarcoma was confirmed on central histology review and classified by a modification of the system proposed by Ober (3). Steroid receptor content was assayed wherever possible.

RESULTS

The patient and tumor characteristics are shown in table 1. No significant toxicity was observed. In six patients tamoxifen therapy was discontinued prior to 8 weeks because of rapid unequivocal progression of tumor.

None of 19 patients with uterine leiomyosarcoma (LMS) and three with endometrial stromal sarcoma (ESS) showed a response to tamoxifen. In four patients with LMS there was no change in tumor over periods of time which ranged from 20 to 32 weeks. One of seven (14.3%) patients with a heterologous malignant mixed Müllerian tumor (MMT) has had a partial response. This 82-year-old woman presented with massive intrapelvic tumor involving the bladder base and extending to the pelvic sidewalls and an inguinal lymph node also contained tumor (proven by fine-needle aspiration). She received 5000 rad of external megavoltage radiotherapy to the pelvis with shielding of the inguinal node and comcomitant tamoxifen. The inguinal nodal metastasis completely resolved and the pelvic tumor shrank. This patient is alive and well and continues on tamoxifen 20 months after presentation.

Tumor ER and PR assays were performed in six patients. The level of ER and PR did not correlate with the response to tamoxifen (table 1).

DISCUSSION

Tamoxifen at a dose of 40 mg/day appears to lack specific activity against uterine LMS. It can be stated with 95% confidence that the true response rate does not exceed 18%. It is uncertain whether the stabilization of disease which was observed in four patients with LMS (21%) represents antitumor effect. One of seven patients

[1]Received Sept 18, 1985; revised Dec 31, 1985; accepted Jan 21, 1986.

[2]*Coordinating Committee:* R. M. Rome, J. J. Campbell, T. I. Cope, B. L. Hillcoat, D. W. Fortune, R. S. Planner, and W. Morgan. *Participating Institutions and Contributors:* Ludwig Institute for Cancer Research (Prof. M. Tattersall), Royal Brisbane Hospital (M. Webb), Royal Adelaide Hospital (M. Davy), Geelong Hospital (G. O. Smith), Mercy Maternity Hospital (R. Planner), Peter MacCallum Hospital (J. Campbell), Queen Victoria Hospital (A. Day), Royal Women's Hospital (R. M. Rome), King Edward Memorial Hospital (A. McCartney). *Pathology Reviewers:* D. W. Fortune, R. A. Osborn, D. Buntine, J. A. Kirkland, and R. Barter.

[3]We thank ICI Pharmaceuticals for the provision of tamoxifen for this study.

[*]*Reprint requests to:* Dr. Robert M. Rome, Royal Women's Hospital, Grattan St, Carlton 3053, Victoria, Australia.

TABLE 1.—Patient characteristics (n = 29)

Characteristic	No. of patients
Median age in yrs (range): 56 (44–80)	
Median ECOG performance status (range): 1 (0–3)	
Current extent of disease	
Advanced*	15
Recurrent	14
Median interval from diagnosis to recurrence in mos (range): 12 (3–48)†	
Prior treatment	
None	20
Doxorubicin (alone or in combination)	5
Doxorubicin (alone or in combination) and radiotherapy	2
Radiotherapy only	2
Sites of measurable tumor‡	
Pelvis	15
Lung	12
Nodes (including retroperitoneal)	11
Liver	4
Abdomen	3
Bone	3
Pleural effusion	2
Bladder	1
Extradural	1
Histologic subtype	
LMS	19
Heterologous MMT	5
Homologous MMT	2
ESS	3
Response to tamoxifen and steroid receptor content	
Partial response§	1
Progressive disease¶	5

*International Federation of Gynecology and Obstetrics stage III or IV.
†14 patients.
‡19 of 29 patients had measurable tumor in > 1 site.
§ER, < 5 fmols/mg of protein; PR, 22 fmols/mg of protein.
¶ER, ≤ 201 fmols/mg of protein; PR, ≤ 58 fmols/mg of protein.

(14%) with uterine MMT had objective response, and accrual of patients with MMT, ESS, and other types of uterine sarcoma is continuing. The very poor survival of patients with uterine sarcoma underscores the need for continuation of the quest for identification of active and nontoxic agents against these tumors.

REFERENCES

1. SOPER JT, MCCARTY KS, JR, HINSHAW W, ET AL. Cytoplasmic estrogen and progesterone receptor content of uterine sarcomas. Am J Obstet Gynecol 150:342–348, 1984.
2. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.
3. OBER WB. Uterine sarcomas; histogenesis and taxonomy. Ann NY Acad Sci 75:568, 1959.

Phase II Trial of *N*-Methylformamide in Patients With Advanced Colorectal Carcinoma[1]

Kurt Tauer, Nancy Kemeny,* Edgar Cheng, Phyllis Hollander, and Nancy Geller[2]

N-Methylformamide (NMF) is the most active antitumor agent of the *N*-alkyl formamides (1,2). Polar-planar compounds such as NMF may induce differentiation of malignant cells (3). Rather than being cytotoxic, this therapy could convert malignant cells to a more benign phenotype. Activity against xenografts, such as the CX-1 colon tumor, as well as certain cell lines (murine Sarcoma 180, M5076 ovarian sarcoma, and Ehrlich ascites carcinoma) has been documented (4,5). Previous phase I trials suggested a dose ranging from 800 to 1200 mg/m^2 given over 5 consecutive days, with hepatotoxicity being the dose-limiting toxic effect. A phase II trial of NMF in patients with advanced, measurable colorectal adenocarcinoma forms the basis of this report.

METHODS

The dose of NMF was 1000 mg/m^2 iv daily over 20–45 minutes for 5 consecutive days every 4 weeks. However, due to unacceptable hepatotoxicity, only five patients were treated with this dose and the remaining patients received 900 mg/m^2. All patients with advanced colorectal carcinoma, pathologically confirmed at Memorial Hospital, were eligible if the following criteria were met: Karnofsky performance status > 50%; estimated survival of at least 2 months; wbc count > 4000/mm^3; platelet count > 130,000/mm^3; serum bilirubin < 1.0 mg/100 ml; serum alkaline phosphatase < 600 U/L; and serum creatinine < 1.5 mg/100 ml. Screening blood tests were collected each day of treatment to monitor changes in liver function. All patients had bidimensionally measurable disease. Standard definitions of response were used. Reassessment of measurable disease was done monthly. Therapy was continued until progression of disease or intolerable toxicity was seen.

NMF was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, in 2-ml ampules (400 mg/ml of 0.9% NaCl).

RESULTS

Eighteen patients were treated with NMF (table 1). Sixteen patients were evaluable for response, one had an early death, and one was lost to follow-up. Fourteen of these patients were previously untreated. No objective responses or reductions in carcinoembryonic antigen levels were observed.

The dose-limiting toxic effect was hepatotoxicity, ranging from asymptomatic biochemical changes to jaundice. Changes in the liver function tests could be seen as early as Day 3 or Day 4 of the treatment cycle, with peak elevations noted by Days 5–7. These abnormalities were reversible, with return to pretreatment levels by Day 14 of the cycle. Autopsy material available on two patients demonstrated central lobular necrosis of the liver consistent with drug toxicity.

Other toxic effects included gram-negative septicemia, which developed in two patients with normal blood cell counts on Days 5–6 of the treatment cycle; in one patient this occurred each of the three times she was treated with NMF. Nausea and vomiting occurred in 16 patients, severe enough to require hospitalization for hydration in five. Hypophosphatemia (< 2.5 mg/100 ml) was seen in 13 patients, with seven having levels < 2.0 mg/100 ml. Myelosuppression was not observed.

After progression on NMF, four of eight patients receiving conventional chemotherapy had some clinical response (two partial responses and two minor responses).

DISCUSSION

NMF at 900–1000 mg/m^2 daily for 5 days produced no objective tumor regression in 16 evaluable patients with advanced colorectal carcinoma (95% confidence limits, 0%–18%). All patients evaluable for response to NMF had progression of disease within 90 days. The absence of disease stabilization in this patient population argues

[1]Received June 25, 1985; revised Jan 8, 1986; accepted Jan 22, 1986.

[2]Solid Tumor Service (*K. Tauer, N. Kemeny, E. Cheng,* and *P. Hollander*), and Department of Biostatistics (*N. Geller*), Memorial Sloan-Kettering Cancer Center, New York, NY.

* *Reprint requests to:* Dr. Nancy Kemeny, Solid Tumor Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Patient characteristics and toxicity*

Characteristic	
Total	18
Male/female	9/9
Evaluable for response	16
Evaluable for toxicity	18
Median age in yrs (range)	57 (41-74)
Median performance status (range)	80% (60%-100%)†
Median No. of cycles (range)	2 (1-4)
Total No. of courses given	31
Site of disease	
Liver	8
Lung	9
Intra-abdominal	3
Median biochemical changes by Day 5 (range)	
Bilirubin	1.6 (0-4) × entry level
SGOT	3 (0-9) × entry level
Alkaline phosphatase	1.4 (0-2) × entry level
LDH	1.2 (0-2) × entry level
Toxicity	
Nausea/vomiting	14
Hypophosphatemia	13
Nonspecific muscle aches	6
Fatigue	3
Diarrhea	2
Malaise	2

* Unless otherwise specified, values = No. of patients.
†16 patients had a performance status of 80%.

against the possible cytostatic nature of NMF at this dose and schedule.

REFERENCES

1. GESCHER A, GIBSON NW, HICKMAN JA, ET AL. *N*-Methyl formamide: antitumor activity and metabolism in mice. Br J Cancer 45:843-850, 1982.
2. MYERS WPL, KARNOFSKY DA, and BURCHENAL JH. The hepatotoxic action of *N*-methyl formamide in man. Cancer 9:949-954, 1956.
3. SPREMULLI EN, and DEXTER DL. Polar solvents: a novel class of antineoplastic agents. J Clin Oncol 2:2227-2241, 1984.
4. Clinical Brochure. *N*-Methyl formamide (NMF) NSC-3051. Bethesda, MD, National Cancer Institute, June 1982.
5. CLARK DA, PHILIPS FS, STERNBERG SS, ET AL. Effects of *N*-methyl formamide and related compounds in mouse Sarcoma 180 (20590). Proc Soc Exp Biol Med 84:203-207, 1953.

Weekly Low-Dose Doxorubicin in Advanced Breast Cancer[1]

Pol Specenier* and José Thomas[2,3]

It has been reported that doxorubicin, one of the most effective drugs in advanced breast cancer, is less toxic (1) but equally effective when administered in a low-dose weekly schedule (2-4).

PATIENTS AND METHODS

Twenty-four patients with advanced breast cancer were treated with doxorubicin at 10 mg/m^2/week. World Health Organization (WHO) criteria were used to evaluate response and toxicity (5).

RESULTS

Pretreatment characteristics and toxicity are shown in table 1. In five patients treatment failure occurred after three cycles or less. No complete or partial response was observed. Two of three patients who failed to respond to a low dose responded while receiving 75 mg/m^2 every 3 weeks.

CONCLUSION

At 10 mg/m^2/week, doxorubicin is ineffective in the treatment of advanced breast cancer. Other studies which allege good therapeutic effects from low-dose weekly doxorubicin have used a dose of 20 mg (2-4) or 20 mg/m^2 (1). The generally mild toxicity was probably also related to the very low dose we used.

TABLE 1.—Patient characteristics and toxicity

Characteristic	No. of patients
Total	24
Median age in yrs (range): 53 (34-73)	
Previous adjuvant chemotherapy	11
Previous therapy for metastases	
Castration	5
Tamoxifen	21
Aminoglutethimide	5
CMF*	23
Anthracycline-containing regimens	4
Sites of metastases at start of low dose	
Skin	13
Supraclavicular and/or axillary nodes	8
Bone	21
Pleura	8
Lung	7
Liver	11
Brain	1
Other breast	3
Toxicity	
Hematologic (WHO scale)	
No change or improvement	13
Grade 1	4
Grade 2	4
Grade 3	2
Grade 4	1
Clinical or ECG evidence of cardiac toxicity	0
Gastrointestinal	
Nausea	8
Transient vomiting	3
Vomiting requiring therapy	1
Slight diarrhea	1
Anorexia	1
Slight conjunctivitis	2
Mild stomatitis	1
Minimal hair loss	2

* CMF = cyclophosphamide, methotrexate, and 5-FU.

REFERENCES

1. TORTI FM, BRISTOW MR, HOWES AE, ET AL. Cardiotoxicity of doxorubicin on a weekly schedule. Ann Intern Med 99:745-749, 1983.
2. MATTSON W, BORGSTRON S, and LANDBERG T. A weekly schedule of low dose doxorubicin in treatment of advanced breast cancer. Clin Ther 5:193-203, 1982.
3. GUNDERSEN S, MATTSON W, KVALÖY S, ET AL. Weekly low dose of adriamycin versus VAC in advanced breast cancer. *In* Proceedings of the 13th International Congress of Chemotherapy, Vienna, 1983, vol 279, pp 6-9.
4. JASPERS L, POSSINGER K, and SCHMID L. Weekly administration schedule of doxorubicin in treatment of patients with advanced breast cancer. *In* Proceedings of the 10th Annual Meeting of ESMO, Nice, Dec 1984, Abstr 139.
5. World Health Organization Handbook for Reporting Results of Cancer Treatment. Geneva, WHO Offset Publication, 1979, pp 22-24.

[1]Received July 12, 1985; revised Nov 20, 1985; accepted Jan 30, 1986.

[2]Department of Oncology, University Hospital St. Rafaël, Leuven, Belgium.

[3]We thank Mrs. K. Vantongelen for the data handling and Mrs. L. Minnen for the typing of the manuscript.

* *Reprint requests to:* Pol Specenier, MD, Department of Oncology, University Hospital St. Rafaël, Capucijnenvoer 33, 3000 Leuven, Belgium.

Intrinsic Versus Acquired Drug Resistance

Goldie and Coldman's mathematical model of the origin of drug resistance, recently restated in this journal (1), provides a valuable conceptual framework for clinicians and biologists attempting to rationalize the problem of the unresponsive tumor. The model certainly gives a plausible explanation for tumor regrowth after an initial response to therapy, but does it really provide a satisfactory explanation for primary treatment failure? The spontaneous mutation model is internally logical, but the authors' assertion (2) that "the major impediment to more successful cancer chemotherapy is the clonal heterogeneity of tumors arising as a consequence of genetic variability" is unsubstantiated and may well be incorrect.

The problems lie not in the authors' deductions but in the premises on which the model is based. One fundamental difficulty is the assumption that in its natural condition the tumor cell is "sensitive," and "resistance" is only subsequently acquired through stable heritable changes occurring during the evolution of the tumor. The use of these qualitative terms seems to have obscured the need for a quantitative description of the drug sensitivity of the "wild type" tumor cell which, in the absence of selective pressure, forms the dominant population. The authors acknowledge that growth kinetics and cellular environment may influence responsiveness, but *intrinsic* variations in cellular chemosensitivity are not usually considered. Until recently, radiobiologists neglected intrinsic radiosensitivity in favor of cellular microenvironment in their explanation of the variation in radiation response among different tumor types. A recent reappraisal of a large volume of radiation survival data, however, shows that, when radiation survival curves are carefully analyzed, they reveal very large variations in radiation sensitivity among tumor cell lines. These variations strongly correlate with the clinical radiation sensitivity of the tumor of origin (3). The main difference is in the "shoulder" of the survival curves, which is thought to reflect the efficiency of cellular repair processes.

The well-recognized correlation between the radiation sensitivity and the chemosensitivity of different classes of human tumor suggests the unifying hypothesis that intrinsic drug sensitivity and intrinsic radiation sensitivity have a common basis which may depend on the efficiency of cellular repair processes. Thus, there is every reason to look again at cellular chemosensitivity data to assess the importance of intrinsic drug resistance in determining the outcome of clinical treatment. The clonogenic assay describes cellular response to cytotoxic agents over a range of only one or two logarithms and is unable to detect or describe the properties of minor resistant subpopulations. This technique thus looks only at the properties of the dominant population which, in the case of an untreated tumor, may be equated with intrinsic chemosensitivity. The strong correlation between cellular chemoresistance in the clonogenic assay and initial resistance to chemotherapy in the patient (4) thus makes it quite unnecessary to invoke cellular heterogeneity as the explanation for initial treatment failure. Tumor *response*, as the term is used clinically, is a function of the properties of the dominant cell population, although the probability of *cure* following an initial response may indeed be a function of the most resistant subpopulation.

While the early use of multiple non-cross-resistant cytotoxic agents is a useful strategy to circumvent the problem of acquired drug resistance, there is no reason to believe that it will be of any value when the dominant cell population is intrinsically resistant. Rather than persisting with an approach which has not been useful in dealing with clinically unresponsive tumors, it may now prove more profitable to investigate the difference between responsive and resistant tumors in the hope that an understanding of the mechanism of intrinsic resistance will ultimately suggest the means to combat it. Although experimental studies of acquired drug resistance are producing valuable results (5), there is currently no evidence that intrinsic resistance has a similar basis and, for the moment, it ought to be treated as a separate phenomenon.

REFERENCES

1. COLDMAN AJ, and GOLDIE JH. Role of mathematical modeling in protocol formulation in cancer chemotherapy. Cancer Treat Rep 69:1041-1045, 1985.
2. GOLDIE JH, and COLDMAN AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. Cancer Res 44:3643-3645, 1984.
3. FERTIL B, and MALAISE EP. Intrinsic radiosensitivity of human cell lines is correlated with radioresponsiveness of human tumors: analysis of 101 published survival curves. Int J Radiat Oncol Biol Phys 11:1699-1707, 1985.
4. SALMON SE, HAMBURGER AW, SOEHNLEN B, ET AL. Quantitation of differential sensitivity of human tumor stem cells to anti-cancer drugs. N Engl J Med 298:1321-1327, 1978.
5. LING V, and THOMPSON LH. Reduced permeability in CHO cells as a mechanism of resistance to colchicine. J Cell Physiol 83:103-116, 1974.

William J. Mackillop[1,*]

November 20, 1985

[1]Ontario Cancer Treatment and Research Foundation, Kingston Regional Cancer Centre; and Cancer Research Laboratories, Department of Pathology, Queen's University, Kingston, Ontario, Canada.

[*]*Reprint requests to:* Dr. William J. Mackillop, Ontario Cancer Treatment and Research Foundation, Kingston Regional Cancer Centre, King St West, Kingston, Ontario, Canada K7L 2V7.

Reply

Dr. Mackillop questions the validity of the somatic mutation model to adequately account for instances of primary treatment failure in cancer chemotherapy and suggests that an analogy with radiation therapy in this context may be more relevant.

We agree that the question of so-called intrinsic resistance to specific agents is an important one, and it should be a goal to understand both the general processes that result in this condition and the specific biochemical mechanisms that appear to be operating at the level of individual cells.

It would be useful to begin with an appropriate definition of intrinsic resistance. Assessment of resistance using purely clinical parameters is fraught with difficulties. There is much evidence to suggest that the degree of clinical response tends to significantly underestimate the actual cell kill achieved with a cytotoxic agent. In experimental systems even simple stabilization of disease without actual regression may be associated with a significant antitumor effect. It is possible, therefore, that many instances of refractory tumors may still be characterized by a sensitive (S) to resistant (R) cell ratio that is much greater than 1. It is true that clonogenic assays do suggest instances where a tumor appears to be composed of 100% resistant cells (without having undergone prior selection). Even these instances must be interpreted with some caution, as (*a*) the tests are usually carried out with single agents and (*b*) refinements in methodology (large colony cut-off technique) may require significant modification of our quantitative data on in vitro cell killing effects (1).

Nonetheless, if we concede that there are numerous instances where all of the clonogenic cells in a tumor are drug resistant, how can we best account for this phenomenon? It should be remembered that intrinsic resistance to one or indeed several types of antineoplastic agents does not necessarily imply resistance to all. Even quite responsive classes of tumor (ie, germ cell tumors) do not display uniform sensitivity to the entire spectrum of anticancer drugs. A model of intrinsic resistance that implied total resistance to all agents beginning from the time of initial transformation would require that the initial tumor cell already be resistant to the 40 or more clinically available antineoplastic agents and indeed the several thousand other agents that have been found in test systems but have not been followed through as clinically exploitable agents. In other words, the initial single cell would express the same amount of information regarding drug resistance as would an entire mature tumor of 10^{11} cells. This is not an impossible situation but begins to appear less likely when viewed in these explicit terms.

In point of fact, it is quite simple to account for a neoplasm that is essentially 100% resistant at the time it has evolved to a mature size, even if the initially transformed cell was sensitive to a given class of antineoplastic agents. This can be done within the context of the somatic mutation model and is achieved by simply permitting the resistant mutant to have a slight growth advantage (either shorter intermitotic time or higher self-renewal probability) as compared to the sensitive phenotypes. With quite minor parameter adjustments this can yield a tumor that is composed of 10% to > 99% resistant clonogenic cells when it has reached a mature size, and without invoking unrealistically high mutation rates (2).

It might seem, at one level, that debate regarding this issue is somewhat fruitless because either model can yield a mature tumor that is refractory to treatment and certainly incurable. There is, however, an important distinction between some of the predictions that the intrinsic resistance as opposed to the acquired resistance model generates. This has to do with the potential curability of very small tumor burdens. If malignancies such as colonic adenocarcinoma are resistant to essentially all classes of cytotoxic agents, even when they consist of one or few cells, then adjuvant chemotherapy directed at patients with minimal tumor burdens is not going to be effective. In contrast, an acquired resistance model, even if it yields situations where the minimal tumor burden is sensitive to only one or two agents, does open up the possibility for effective adjuvant chemotherapy programs, providing it is directed at patients who have truly minimal tumor burdens.

We would suggest that this appears to be the most important testable difference between the two concepts. The curable burdens for tumors such as colonic carcinoma and melanoma may be very low, but need not be zero. Optimally delivered high-dose intensity programs incorporating 5-FU and dacarbazine may make an impact on favorable subsets of these tumors. This seems to us an important question deserving serious study.

REFERENCES

1. BUICK RN. The cell renewal hierarchy in ovarian cancer. *In* Human Tumor Cloning: Proceedings of the 4th Conference on Human Tumor Cloning, Tucson, AZ (Salmon SE, and Trent JH, eds). New York, Grune and Stratton, 1984, pp 3–13.
2. COLDMAN AJ, GOLDIE JH, and NG V. The effect of cellular differentiation of the development of permanent drug resistance. Math Biosciences 74:177–198, 1985.

James H. Goldie*
Andrew J. Coldman[1]

January 9, 1986

[1]Cancer Control Agency of British Columbia, Vancouver, Canada.

*Reprint requests to: James H. Goldie, MD, Cancer Control Agency of British Columbia, 600 W 10th Ave, Vancouver, British Columbia, Canada V5Z 4E6.

Simple Method for the Administration of High-Dose Etoposide During Autologous Bone Marrow Transplantation[1]

Etoposide is an effective anticancer agent, but when used in high dose it may require considerable expertise. We developed a simple system which allows easy delivery of large amounts of etoposide in the course of autologous bone marrow transplantation. Seven cases are presented in which high-dose etoposide was given through a central venous catheter without problems.

Etoposide is a semisynthetic podophyllotoxin derivative which is active in standard dose against a number of solid tumors and hematologic malignancies (1,2). Due to erratic absorption, etoposide usually is given by the iv route (1,3). Recently, several investigators have administered significantly higher doses of drug in an attempt to increase the antitumor effect of this agent (4,5). Because of instability in solution, it is recommended that etoposide be diluted in normal saline or 5% dextrose in water at a concentration of 0.2–0.4 mg/ml; concentrated solutions of ≥ 1 mg/ml show crystal formation within 5 minutes upon stirring or 30 minutes after standing (3). Thus, at the higher concentrations the amount of active drug infused may be less than the calculated dose. Doses up to 3500 mg/m^2 can be given within a few days, and large volumes of saline or fluid must be given concomitantly, with the risk of precipitating congestive heart failure; also, a great deal of labor, time, and cost is expended to prepare the infusions, which may need to be administered hourly (4,5). We report that etoposide can be given easily and safely through a central venous catheter connected to a simple pump system which avoids these problems.

Seven patients were entered into phase I–II studies which used high-dose etoposide and autologous bone marrow transplantation. These studies were approved by the Institutional Review Board for Human Investigation at the University Hospitals of Cleveland, and patients gave written informed consent. Etoposide undiluted from the vial (20 mg/ml) was obtained from Bristol-Myers Co, and for the first patient (No. 1) etoposide was drawn into two 30-ml plastic syringes and directly connected to an IMED infusion pump (IMED Corporation, San Diego, CA).

For the subsequent patients treated (Nos. 2–7), etoposide was drawn into two or three 30- or 50-ml plastic syringes using sterile technique and placed sequentially into a Sage Instrument syringe pump model 341A (Orian Research Inc, Cambridge, MA).

The IMED tubing or syringe pump tubing was connected to a double lumen indwelling central venous catheter (Cook Inc, Bloomington, IN) and the etoposide was infused iv over 3–4 hours. The actual dose of drug infused per day ranged from 960 to 2700 mg (table 1). The infusion rate was 0.34–0.67 ml/min, or 6.8–13.4 mg/min of etoposide (table 1). Afterward, 30 ml of normal saline was infused to flush the iv tubing.

DISCUSSION

We report that etoposide in high dose can be given safely iv through a central venous catheter to avoid many of the problems associated with preparation and adminis-

[1]Supported in part by a grant from the Diamond Shamrock Chemicals Company.

TABLE 1.—Patient characteristics and etoposide infusion data

Patient No.	Diagnosis*	Age (yrs)	Regimen	Actual drug dose (mg/day)	Drug infusion rate (ml/min)
1	NSCCL	63	600 mg/m^2 × 3 days	1200	0.34
2	NSCCL	59	600 mg/m^2 × 3 days	960	0.34
3	NSCCL	68	600 mg/m^2 × 3 days	1260	0.34
4	ANLL	44	50 mg/kg × 1 day	2700	0.67
5	ANLL	49	50 mg/kg × 1 day	2700	0.67
6	NSCCL	62	600 mg/m^2 × 3 days	1200	0.34
7	NSCCL	64	600 mg/m^2 × 3 days	1200	0.34

*NSCCL = non-small cell carcinoma of the lung; ANLL = acute nonlymphocytic leukemia.

tration of large quantities of this drug. Other investigators infuse high-dose etoposide diluted in normal saline (final concentration, 0.8–1.0 mg/ml) over several hours (4,5). The dilution method results in the delivery of large volumes of saline over a short period of time (increasing the risk of cardiac dysfunction and fluid and electrolyte problems) and may require hourly preparation of reagents. A marked savings in labor, time, and cost can be accomplished using our infusion protocol.

Problems such as hypotension and bronchospasm that have been reported with rapid infusion of etoposide were not observed in the seven patients we studied (1). Similarly, no side effects such as acidosis due to the complex drug solvent system (polyethylene glycol, Tween 80, citric acid, and absolute ethanol) nor difficulties in infusing the highly viscous etoposide concentrate through a small-bore (0.04-inch inside diameter) central venous catheter were noted (5). The syringe pump system, however, seemed more easily adaptable to the clinical situation than the IMED pump, since there were fewer tubing connections, and less plasticware was required. Our simple method for the administration of high-dose etoposide may facilitate the use of this highly effective antitumor agent in other clinical situations.

REFERENCES

1. VOGELZANG NJ, RAGHAVAN D, and KENNEDY BJ. VP-16-213 (etoposide): the mandrake root from Issyk-Kul. Am J Med 72:136–144, 1982.
2. O'DWYER PJ, LEYLAND-JONES B, ALONSO MT, ET AL. Etoposide (VP-16-213). Current status of an active anticancer drug. N Engl J Med 312:692–700, 1985.
3. PHILLIPS NC, and LAUPER RD. Drug reviews. Review of etoposide. Clin Pharm 2:112–119, 1983.
4. POSTMUS PE, MULDER NH, SLEIJFER DT, ET AL. High-dose etoposide for refractory malignancies: a phase I study. Cancer Treat Rep 68:1471–1474, 1984.
5. WOLFF SN, FER MF, MCKAY CM, ET AL. High dose VP-16-213 and autologous bone marrow transplantation for refractory malignancies: a phase I study. J Clin Oncol 1:701–705, 1983.

Hillard M. Lazarus*
Richard J. Creger
Diana Diaz[2]

December 3, 1985

[2]Departments of Medicine and Pharmacy Services, (*H. M. Lazarus* and *R. J. Creger*) and Department of Nursing (*D. Diaz*), Case Western Reserve University, University Hospitals of Cleveland, OH.

Reprint requests to: Hillard M. Lazarus, MD, Department of Medicine, University Hospitals of Cleveland, 2074 Abington Rd, Cleveland, OH 44106.

Dose-Limiting Myelosuppression Associated With High-Dose Cisplatin (200 mg/m^2) in Hypertonic Saline

The rationale for high-dose cisplatin in hypertonic saline is based on reducing dose-limiting nephrotoxicity by changing the vehicle of administration, as well as the potential dose-response relationship for this agent. Using 4.5% saline as the vehicle of administration for cisplatin, Litterst (1) demonstrated protection against nephrotoxicity in a mouse model without loss of antitumor effectiveness. The initial clinical trial reported by Ozols et al (2) utilized this concept to administer cisplatin in a total dose of 200 mg/m^2 given in a 5-day regimen (40 mg/m^2/day as a 30-minute infusion), with reported increased efficacy in poor-prognosis testicular cancer and responses in ovarian cancer refractory to standard-dose cisplatin. In this study and in subsequent reports, although renal toxicity has been mild, myelosuppression and neurotoxicity have been severe and have become the dose-limiting toxic effects (3). In a recent report of cisplatin pharmacokinetics, both improved tumor efficacy and the severity of toxicity were attributed to a threefold increase in plasma concentration and systemic exposure (4). In many of these studies, the specific contribution of cisplatin to the severity and pattern of toxic effects has been difficult to determine, since combination chemotherapy regimens were used or patients had received prior chemotherapy.

In a feasibility study, we have evaluated the use of single-agent high-dose cisplatin in hypertonic saline in advanced non-small cell lung cancer (5). Our dose schedule is similar to that previously described (40 mg/m^2/day for 5 days) with the exception of an infusion time of 4 hours and a reduction in hydration requirement to 150 ml/hour. A total of 16 cycles were administered to seven patients. Similar to previous reports, renal toxicity was relatively mild, with no patient developing an increase in serum creatinine > 0.8 mg/100 ml. Even as a single agent, however, myelosuppression was more severe than usually observed following standard-dose cisplatin. Thrombocytopenia (< 25,000 cells/mm^3) occurred in two patients, with a nadir at Day 18. Both patients received prophylactic platelet transfusions. Granulocytopenia (< 1500 cells/mm^3) was also seen in two patients, with a delayed nadir occurring at approximately Day 25. No patient developed bleeding or treatment-related infection. With the 4-hour infusion time, clinically apparent peripheral neuropathy or hearing loss was not observed. One complete remission and one partial remission were seen in two of six evaluable patients.

In prior studies, cisplatin has often been combined with other agents, and patients in some series have received prior chemotherapy which includes neurotoxic agents. These factors are of special importance in considering the severity of myelosuppression and the degree of neurotoxicity previously described. In our feasibility study, high-dose cisplatin was administered as a single agent, and no

patient had received prior therapy. Myelosuppression, however, was still severe in some patients and appears to be the dose-limiting toxic effect of this modified regimen. Significant neurotoxicity was not apparent in our study. It is unclear whether this reflects the small patient sample size or whether this may relate to the prolonged infusion time of 4 hours. If neurotoxicity is related to peak plasma levels of cisplatin, then increasing infusion time from 30 minutes to 4 hours may account for this difference in observed toxicity.

In summary, the dose-limiting toxic effect of single-agent high-dose cisplatin as delivered in this study appears to be myelosuppression, predominantly thrombocytopenia. This pattern of myelosuppression should be considered when designing combination chemotherapy regimens using this type of high-dose regimen. The efficacy of high-dose cisplatin in tumor types such as non-small cell lung cancer remains to be determined.

REFERENCES

1. LITTERST CL. Alterations in the toxicity of *cis*-dichlorodiammineplatinum-II and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. Toxicol Appl Pharmacol 61:99–108, 1981.
2. OZOLS RF, CORDEN BJ, JACOB J, ET AL. High dose cisplatin in hypertonic saline. AIM 100:19–24, 1984.
3. WILDING G, GRIFFIN IW, COSTANZA ME, ET AL. Phase I–II trial of non-small cell lung cancer with high dose cisplatin in hypertonic saline. Proc ASCO 3:219, 1984.
4. CORDEN BJ, FINE RL, OZOLS RF, ET AL. Clinical pharmacology of high-dose cisplatin. Cancer Chemother Pharmacol 14:38–41, 1985.
5. MANSOUR R, GANDARA D, WOLD H, ET AL. Myelosuppression associated with ultra high dose cisplatin (200 mg/m^2) in hypertonic saline. Proc ASCO 4:179, 1985.

David R. Gandara*
Richard Mansour
Howard Wold
Christopher George[1]

December 3, 1985

[1]Letterman Army Medical Center, San Francisco, CA; University of California, Davis, and Martinez VA Medical Center, CA.

Reprint requests to: David R. Gandara, MD, University of California, Davis, Martinez VA Medical Center, 612/111H, 150 Muir Rd, Martinez, CA 94553.

ANNOUNCEMENTS

Cancer Treatment Reports On-Line

The complete text of *Cancer Treatment Reports* is now available in two, user-friendly, commercial databases for medical professionals: BRS/Saunders Colleague and Mead Data Central's MEDIS.

Both databases, which are easily accessible from personal computers equipped with modems, offer full text of a substantial number of medical journals and books, and they also include other medical files such as the National Cancer Institute's PDQ (Physician Data Query) or the National Library of Medicine's MEDLINE.

For details on the information contained in each of the databases, the hardware required to access them, and the subscription fees, we encourage interested readers to contact both vendors: *BRS/Saunders*, 1290 Avenue of the Americas, Suite 1200, New York, NY 10104 (telephone: 1-800-468-0908); *Mead Data Central*, P.O. Box 933, Dayton, OH 45401 (telephone: 1-800-227-4908).

1986 NATO Advanced Study Institute on Physics and Technology of Hyperthermia

Urbino, Italy, July 26–August 9, 1986

This summer school aims to present the basic physics and technology of hyperthermia, together with sufficient clinical and biological background of hyperthermic oncology, to physicists and engineers to allow for competent design and development of methods and systems for experimental research and clinical studies.

The course is intended for advanced graduate students, postdoctoral fellows, and research workers for universities, hospitals, and industry and government laboratories. The number of students admitted will be limited to 80, and a selection of participants will be made to ensure the interdisciplinary character of the Institute. A limited number of grants are available for qualified advanced students, recent graduates, and hospital physicists of developing countries towards travel and living expenses.

For further information, contact: S. B. Field, MRC Cyclotron Unit, Hammersmith Hospital, Ducane Rd, London W12, United Kingdom (telephone: 44-1-743.4594); or C. Franconi, Medical Physics Institute, 11' University of Rome, Via O. Raimondo, 00173 Rome, Italy (telephone: 39-6-613.1200).

Sixth Annual Epidemiology Summer Program

Tufts University, Medford, Massachusetts, July 27–August 15, 1986

The 3-week summer program in epidemiology will be hosted in 1986 by Tufts University at its Medford Campus. The program includes methodologic and substantive courses for those seeking a review of recent developments in epidemiologic thinking. Registrants may receive graduate degree credit, CME credit, and industrial hygiene CM credit. The following courses will be offered: Theory and Practice of Epidemiology, I; Theory and Practice of Epidemiology, II; Theory and Practice of Case-Control Research; Biostatistics for Epidemiologists; Regression and Categorical Data Methods; Nutritional Epidemiology; Microcomputers for Epidemiologists; Data Acquisition and Management; Epidemiologic Basis for Public Policy; Logistic Regression and Survival Analysis; Environmental and Occupational Epidemiology; Cancer Epidemiology; Biomedical Writing; and Reproductive and Perinatal Epidemiology.

Tuition fees are payable to the New England Epidemiology Institute. The fees are as follows: $400 for 1 course; $600 for 2 courses; $800 for 3 courses; + $60 for each course over 3 courses. Courses may be taken for graduate credit (maximum, 2 per course) for an additional charge of $35 per credit.

For further information, contact: The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

International Congress on The Biological and Clinical Aspects of Phagocyte Function

Pavia, Italy, September 7–10, 1986

The Italian Society of Hematology is organizing the International Congress on The Biological and Clinical Aspects of Phagocyte Function to be held in Pavia, Italy, September 7–10, 1986. Topics for discussion include: leukocyte random locomotion, chemotaxis, and chemokinesis; membrane metabolism; pharmacological manipulation and immunomodulation of phagocyte function; genetic control of phagocyte function; the role of phagocyte in inflammation; leukotrienes, prostaglandins, chemotaxis, and phagocyte function; membrane chemotactic and phagocyte function; and the clinical aspects of phagocyte disorders.

The program will include free oral presentations and poster sessions selected from the submitted abstracts. Registration fee after May 31, 1986, is $200 US.

For further information, contact: Giovanni Ricevuti, Istituto di Patologia Speciale Medica 1ª, Università di Pavia, 27100 Pavia, Italy (telèphone: 0382/390590).

35th National Meeting of the Italian Neurosurgical Society

Pavia, Italy, September 18–20, 1986

Topics of discussion at this meeting include cerebral aneurisms, pathology of the cervical column, brain tumors in infancy, spinal tumors, cerebral ischemia, and pain therapy.

For further information, contact: Prof. Pietro Paoletti, Department of Surgery, Neurosurgical Clinic, University of Pavia, San Matteo Hospital, 27100 Pavia, Italy (telephone: 0382/422231).

Nutritional and Pharmacological Treatment of Brain Tumors

Pavia, Italy, September 18, 1986

The International Meeting on Nutritional and Pharmacological Treatment of Brain Tumors will be held in Pavia, Italy, on September 18, 1986, and is a Satellite Meeting of the XXXV Italian Congress of Neurosurgery.

For further information, contact: Prof. Rodolfo Paoletti, Centro Studi Dell'Alimentazione, via S. Pietro all'Orto, 17, 20121 Milan, Italy (telephone: 02/709.514).

Diagnosis and Therapy of Bone Marrow Abnormalities: Cancer and Stem Cell Defects

San Francisco, California, September 25–26, 1986

The Bone Marrow Transplant Unit of the Cancer Research Institute and the Department of Pediatrics of the University of California School of Medicine in San Francisco announce a symposium on diagnostic and therapeutic advances in bone marrow abnormalities. Designed for pediatric and general oncologists, the program will be held at the Sheraton on Fisherman's Wharf in San Francisco, California, September 25–26, 1986. Speakers include members of the Departments of Pediatrics and Medicine (Oncology) of the University of California School of Medicine as well as featured guest faculty. The program will meet the criteria for ACCME/AMA/CMA Category 1 credit.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143, (telephone: 415-476-4251).

Principles of Epidemiology

Cambridge, Massachusetts, October 6–8, 1986

The New England Epidemiology Institute will present a 3-day course on "Principles and Methods of Epidemiology," at the Charles Hotel in Cambridge, MA. The course will be taught by Drs. Kenneth Rothman and Stephen Lanes and by Mr. Charles Poole. The course will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. No previous study of epidemiology or biostatistics is

required. Registrants may receive Continuing Medical Education Credits (AMA Category 1) upon application and/or Certification Maintenance Credits from the American Board of Industrial Hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Mechanisms of Drug Resistance in Neoplastic Cells

Washington, DC, October 15–16, 1986

The Ninth Annual Bristol-Myers Symposium on Cancer Research entitled "Mechanisms of Drug Resistance in Neoplastic Cells" will be held on October 15–16, 1986, at the Washington Hilton, Washington, DC. The meeting is being hosted by the Vincent T. Lombardi Cancer Research Center of Georgetown University. The registration fee is $75 ($20 for students, residents and fellows). Preregistration is required.

For additional information, contact: Mary Day, Office of Continuing Medical Education, Georgetown University Medical Center, 3800 Reservoir Rd, NW, Washington, DC 20007 (telephone: 202-625-2306).

Second International Symposium on Epstein-Barr Virus

St. Petersburg, Florida, October 17–22, 1986

This symposium, dedicated to the memory of Professor Yohie Ito, will cover the current advances in Epstein-Barr virus (EBV)-associated diseases such as Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative syndrome, chronic infectious mononucleosis, and immunodeficiency syndromes including AIDS and organ transplants. The various scientific presentations will include overviews, selected papers, and poster sessions. The program will concentrate on both clinical and basic research aspects. Specifically, the symposium will review the latest advances in disease manifestations associated with EBV infections and will cover clinico-pathologic features, epidemiologic and genetic aspects, and new developments in basic research on EBV including: intraction of oncogenes, EBV genomes, gene expressions, mapping and proteins, cellular immune responses, co-factors associated with EBV infections, and control of EBV infections.

Dr. M. Nonoyama, Showa University Research Institute for Biomedicine in Florida; Dr. R. Glaser, Ohio State University; Dr. G. Pearson, Georgetown University; and Drs. D. Ablashi and P. Levine, National Cancer Institute, Bethesda, MD, are organizing the symposium.

For further information, contact: Dr. Nonoyama, Showa University Research Institute for Biomedicine in Florida, St. Petersberg, FL 33702; or Dr. Ablashi, Bldg 37, Rm 1E24, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (telephone: 301-496-9332).

Fifth Annual Cincinnati Cancer Conference

Cincinnati, Ohio, October 31–November 1, 1986

Breast cancer and cancer nursing will be the focuses of the Fifth Annual Cincinnati Cancer Conference. Cancer specialists from across the country will discuss various modalities in the treatment of breast cancer, including surgery, radiotherapy, new directions in chemotherapy and hormonal therapy, and endocrine treatment. The detection of breast cancer and the role of diet in breast cancer will also be covered.

Sessions devoted to nursing issues will take up various topics, such as: legal and ethical issues in outpatient and home care; the creation of effective interdisciplinary relationships in outpatient and home care; implementation of research in pain control; advanced cancer nursing practice; and a 10-year cancer nursing forecast.

Conference coordinators are Barth Hoogstraten, MD, and Hilary Wood, RN. The conference is sponsored by Bethesda Hospital, Inc, of Cincinnati.

For further information, contact: Thomas O'Connor, Continuing Medical Education, Bethesda Hospital, Inc, 619 Oak St, Cincinnati, OH 45206.

12th Annual Congress of the European Society for Medical Oncology

Nice, France, November 28–30, 1986

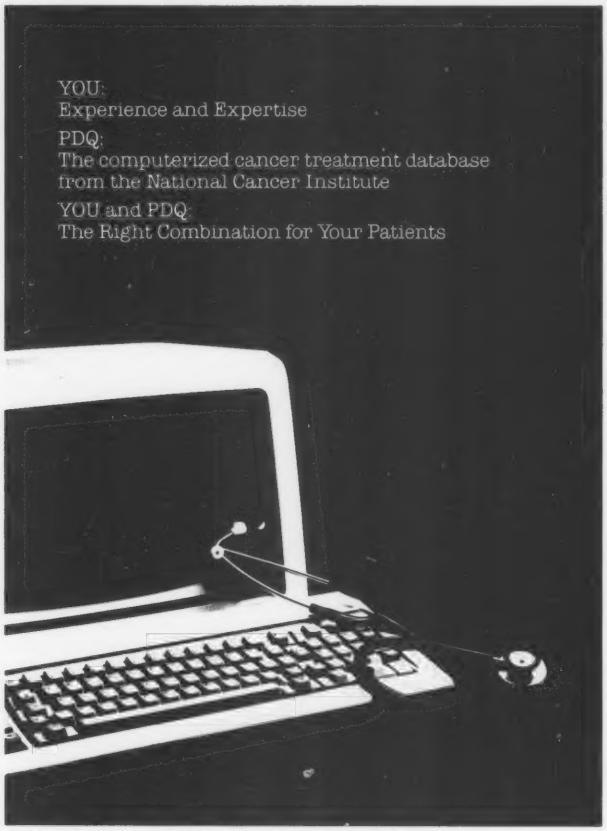
The scientific program for the 12th Annual Congress of the European Society for Medical Oncology will include: 1. Reports on the most important 1985–1986 international meetings devoted to cancer. 2. Nine sessions with oral communications and posters on preclinical and clinical phase I–II studies, biological response modifiers, medical treatments, and therapeutic strategies. 3. Plenary lectures on medical oncology.

For further information, contact: Prof. M. Schneider, Centre Antoine-Lacassagne, 36 Voie Romaine, 06054 Nice Cedex, France (telephone: 93 81 71 33).

35th Annual Meeting of the Radiation Research Society

Atlanta, Georgia, February 22–26, 1987

For further information, contact: Radiation Research Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3153).



The National Cancer Institute, International Cancer Information Center, R.A. Bloch Building, Bethesda, Maryland 20892. (301) 496-7403.

*U.S. GOVERNMENT PRINTING OFFICE: 1986-491-283:10021

An Estimated 2 to 4 Percent of All Cancer Deaths Are Related To Alcohol

The Spring 1986 issue of ***Alcohol Health and Research World,*** the quarterly magazine of the National Institute on Alcohol Abuse and Alcoholism, presents reports by prominent investigators in the field of alcohol and cancer. Original articles describe possible underlying mechanisms by which alcohol may be associated with certain types of cancers, review recent research findings on alcohol as a risk factor for cancer, and provide a discussion of cancer prevention strategies.

Because ***Alcohol Health and Research World*** explains state-of-the-art research findings in language that readers can understand, this special Spring 1986 issue on Alcohol and Cancer can help all readers incorporate alcohol and cancer prevention education messages in their interactions with patients and clients.

To subscribe, fill out the form below and mail it — along with a check, money order or credit card information for $12.00 ($15.00 foreign) — to Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

Alcohol Health and Research World — The link between theory and practice in the alcohol field.

Alcohol Health & Research World

Cancer

Subscription rates are:
$12.00/year domestic
$15.00/year foreign
Please make checks payable to:
Superintendent of Documents

Enclosed is $ _____
☐ Check
☐ Money order
☐ Charge to my deposit account no.

_____-_____

Order No. _____

☐ Credit Card (fill out section below)

Company or Personal Name

Additional Address/Attention Line

Street Address

City State ZIP Code

Country

Telephone Numbers
Area Code Home
Area Code Office

Charge orders may be telephoned to the GPO order desk at (202) 783-3238 from 8:00 am to 4:00 pm eastern time, Monday-Friday (except holidays).

MasterCard and VISA accepted.

Credit Card No.

Expiration Date (month/year)

$ _____
Total Charges

Mail to: Superintendent of Documents
US Government Printing Office
Washington, DC 20402

To: CTR-CAN
SUPERINTENDENT OF DOCUMENTS
U.S. Government Printing Office
Washington, D.C. 20402

Subscription prices:

$29 per year domestic
$36.25 per year foreign

Make checks payable to the Superintendent of Documents

☐ I want to receive my own copy of the *Cancer Treatment Reports* for the next year.

☐ Here's my check for $_____ made out to the Superintendent of Documents.

Name _____

Address _____

City _____ State_____ Zip_____

Charge my credit card ☐ VISA ☐ MasterCard ☐ Deposit Account ☐ Choice

Credit Card No._____

Expiration Date _____

Signature _____

A Publication of the National Cancer Institute

NIH Publication No. 86-22